The Peopling of America: Craniofacial Shape Variation on a Continental Scale and its Interpretation From an Interdisciplinary View

Rolando González-José,[1]* Maria Cátira Bortolini,[2] Fabrício R. Santos,[3] and Sandro L. Bonatto[4]

[1]*Centro Nacional Patagónico, Consejo Nacional de Investigaciones Científicas y Técnicas, U9120ACF Puerto Madryn, Argentina*
[2]*Departamento de Genética, Instituto de Biociências, Universidade Federal do Rio Grande do Sul, 91501-970 Porto Alegre, RS, Brazil*
[3]*Departamento de Biologia Geral, ICB, Universidade Federal de Minas Gerais, 31270-910 Belo Horizonte, MG, Brazil*
[4]*Faculdade de Biociências, Pontifícia Universidade Católica do Rio Grande do Sul, 90610-001 Porto Alegre, RS, Brazil*

ABSTRACT   Twenty-two years ago, Greenberg, Turner and Zegura (*Curr. Anthropol.* 27:477–495, 1986) suggested a multidisciplinary model for the human settlement of the New World. Since their synthesis, several studies based mainly on partial evidence such as skull morphology and molecular genetics have presented competing, apparently mutually exclusive, settlement hypotheses. These contradictory views are represented by the genetic-based Single Wave or Out of Beringia models and the cranial morphology-based Two Components/ Stocks model. Here, we present a geometric morphometric analysis of 576 late Pleistocene/early Holocene and modern skulls suggesting that the classical Paleoamerican and Mongoloid craniofacial patterns should be viewed as extremes of a continuous morphological variation. Our results also suggest that recent contact among Asian and American circumarctic populations took place during the Holocene. These results along with data from other fields are synthesized in a model for the settlement of the New World that considers, in an integrative and parsimonious way, evidence coming from genetics and physical anthropology. This model takes into account a founder population occupying Beringia during the last glaciation 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 circumarctic 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. Am J Phys Anthropol 137:175–187, 2008.   © 2008 Wiley-Liss, Inc.

Greenberg, et al., (1986) proposed an interdisciplinary model regarding the settlement of America based originally on linguistic data and further supported by dental morphology and genetics. Their model suggested that the ancestors of present-day Native Americans would have come from Siberia in three separate migrations at different times. In their view, present-day Amerind-speaking people or Amerindians, who are the native inhabitants of 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 migration would have involved the Na-Dene speakers (e.g., Navajo, Athabaskan), who currently occupy the North Pacific coast, the interior of Alaska, and parts of the southwest USA. The last independent group of people to enter the continent would have been the Eskimo-Aleuts, who nowadays inhabit Arctic and sub-Arctic lands. The first migrants would also have been the producers of the Clovis culture [~12,000 years before present (YBP)] that is observed in some early archeological sites from North America.

Since the publication of Greenberg et al. (1986)'s model, considerable research in several fields has promoted debate around this and subsequent models, and recent genetic and morphological results are of critical importance for the interpretation of the New World settlement.

GENETIC DATA

Initial analysis using partial mitochondrial DNA (mtDNA) data revealed that most present-day Native Americans belong to five distinct haplogroups, designated

A–D, and the more recently described haplogroup X. These lineages are also found in parts of Asia, thus supporting their northeastern Asian origin (Schurr et al., 1990). Although some of the initial studies with this kind of data seemed compatible with a multiple migration hypothesis (Torroni et al., 1992; Horai et al. 1993), this view was challenged by latter investigations using more individuals and alternative statistical approaches (e.g., Merriwether et al., 1995; Bonatto and Salzano, 1997a,b; Stone and Stoneking, 1998). Furthermore, recent studies analyzing complete mtDNA genomes confirmed the presence of Native American autochthonous subhaplogroups A2, B2, C1, D1, and X2a, as well as their derived lineages (e.g., C1b, C1c, and C1d), reflecting the accumulation of several specific mutations in these populations (Tamm et al., 2007; Fagundes et al., 2008).

These results imply that Asian migrants were isolated somewhere during an extended period of time (likely >5,000 years), long enough to allow the origin of autochthonous lineages and before entering the empty lands of the New World (Bonatto and Salzano, 1997a; Tamm et al. 2007; Fagundes et al., 2008). Archeological and paleoclimatic evidence indicates that this preliminary formation of the American gene-pool occurred in Beringia, which toward the end of the last glacial maximum should have acted as a climatic and ecological refuge. These recent mtDNA genome datasets point to a pre-Clovis occupation of the continent (>15,000 YBP), and refute a separate origin for the three main Native American linguistic groups, while also suggesting a rapid settlement by a Pacific coastal route (Tamm et al., 2007; Fagundes et al., 2008).

Seminal studies on Y-chromosome variation showed the presence of a lineage now recognized as haplogroup Q (YCC, 2002), which occurs in the majority of indigenous individuals in North, Central, and South America (Pena et al., 1995; Santos et al., 1995; Underhill et al., 1996). Its ancestral Asian lineage has been traced back to south-central Siberia (Santos et al., 1999; Karafet et al., 1999; Bortolini et al., 2002, 2003). Haplogroup Q is further divided into subhaplogroups Q3 and Q* (YCC, 2002), the former being considered an autochthonous American chromosome. As haplogroup Q3 represents the immense majority of all native Y chromosomes in America (>80%), including tribes of all major linguistic and cultural groups, this finding indicates the existence of a founder effect during the initial peopling, as well as a common origin for all Native Americans, including Amerindians, Na-Dene, and Aleut-Eskimos (Tarazona-Santos and Santos, 2002; Zegura et al., 2004).

Note that these observations are in agreement with the recent mtDNA results. Dating the first entry into America has been approached through the analysis of Y-microsatellite diversity (Bortolini et al., 2003; Zegura et al., 2004) or Y-SNPs (single nucleotide polymorphisms) and coalescence simulations (Hammer and Zegura, 2002). The resultant range for the first entry data has been point estimated between 14,000 and 18,000 YBP (but with large confidence intervals). These estimates are somewhat more recent but still compatible with the estimates from the mtDNA data presented above.

Recently, an allele (9AR) at autosomal locus D9S1120 was described as highly frequent among all Native American populations and absent from 49 other worldwide populations (Schroeder et al., 2007; Wang et al., 2007). This particular distribution is most consistent with the hypothesis that all modern Native Americans derive from a common founding population (Schroeder et al., 2007), which is also the same scenario depicted by the uniparental loci studies.

Additionally, the genetic data suggest some level of gene flow back from Beringia to western Siberia and/or more recent (but constant) migrations across the circum-Arctic areas. For instance, autochthonous Native American mtDNA and Y-chromosome lineages, as well as the 9AR autosomal allele, are found in low frequencies in Asian populations, especially in northwestern Siberia (Tarazona-Santos and Santos, 2002; Bortolini et al., 2002, 2003; Schurr, 2004; Schroeder et al., 2006; Tamm et al., 2007; Wang et al., 2007).

In summary, recent genetic analyses seem to converge on a single, although complex, scenario for the origin of Native Americans, indicating pre-Clovis times for the first settlement and a crucial role of Beringia during the early phases of occupation. Some gaps still remain, such as the exact timing of the settlement, the number of founders, and the extent of postsettlement gene flow between Asia and America. To address these issues, more refined analyses based on many more markers are needed to estimate more accurate dates and to investigate several putative demographic scenarios.

Morphological data

Previous studies indicated high levels of within and between group heterogeneity in Asia during the late Pleistocene (Lahr, 1996; Brown, 1999; Cunningham and Wescott, 2002; Cunningham and Jantz, 2003) and across the entire Holocene in the New World (González-José et al., 2001, 2003). When considering the total craniofacial diversity within anatomically modern humans, the late Holocene northeastern Asian morphology can be viewed as a set of derived traits, including short and wide neurocrania, high, orthognatic and broad faces, and relatively high and narrow orbits and noses (Lahr, 1996). Even when considerable within and between-group variation is found in these traits, the simultaneous occurrence of this set of characters was classically subsumed (somewhat simplistically) in a single label: the mongoloid skull. Some authors such as Turner (1989), Lahr (1996), Lahr and Foley (1998), Brown (1999), and Neves et al. (2003, 2005) stated that Southeast Asia could be the place of origin of Asian groups who further expanded across East Asia during the last glaciation.

To Howells (1973) and Roseman (2004), the emergence of specialized northeastern Asian traits departing from the generalized late Pleistocene Southeast Asian morphology is likely due to adaptation to cold environments. In this context, Harvati and Weaver (2006) suggest that, while cranial morphology retains a population history signal that tracks neutral genetics well, human facial shape appears to retain a climatic, rather than a genetic, signature. They further state that this climatic effect may be confined to arctic populations.

Despite the broad geographic dispersal of the derived traits in modern northeast Asian groups, people carrying the complete set of traits did not appear until the Neolithic period at sites like Baoji, around 7,800 YBP (Brown, 1999). In fact, late Pleistocene Asian remains are noticeably out of the range of craniofacial variation of modern eastern Asians (An, 1991; Cunningham and Jantz, 2003). Moreover, in their analysis of within-group variation, Cunningham and Wescott (2002) demonstrated

that the Upper Cave specimens (Zhoukoudian, China, 11910 cal. YBP) show significantly more variation than do individuals within many modern human populations.

Although the dating and mode of evolution from a generalized late Pleistocene Southeast Asian to the derived set of modern northeastern Asians traits are not precise, the initial migration to America undoubtedly preceded this specialization. By and large, the most valuable materials concerning early stages in America, in terms of antiquity and sample size, are the skeletal remains from Lagoa Santa, Brazil, dated as earlier as 13,500 YBP (Neves and Hubbe, 2005). Comparative analyses of these remains (Neves and Pucciarelli, 1991; González-José et al., 2001, 2003; Neves and Hubbe, 2005) furnished three important observations. First, the Lagoa Santa samples tended to have low affinities with modern East Asians and Amerindians. Second, the transition to the set of derived features observed after the middle Holocene (~7,000 YBP) in central Brazil seemed to be a sudden process and was suggested to be associated with a dispersal from Asia than with *in situ* evolution (Neves and Hubbe, 2005). Finally, the two previous observations seemed also to occur in other regions of the New World, such as the Central Valley of Mexico (González-José et al., 2005), Patagonia (González-José et al., 2001), southern Chile (Neves et al., 1999a; Mena et al., 2003), and North America (Steele and Powell, 1992, 1993; Brace et al. 2001; Jantz and Owsley, 2001).

Considering North American remains, studies made by Steele and Powell (1992, 1993), and Jantz and Owsley (2001) show that, in agreement with the South American findings, the transition between the early and late Holocene is characterized by a marked morphological discontinuity. Furthermore, the high levels of variability found among the sparse early North American remains suggest that the ancestral population, which first settled the continent, was also highly heterogeneous or else that several migration events occurred during the early phases of occupation (Jantz and Owsley, 2001).

In summary, craniofacial studies seem to support a scenario in which America was successively occupied by two morphologically differentiated human populations, with the generalized (e.g., Paleoamerican) morphology first entering the New World and being replaced or assimilated by groups carrying derived traits (Neves and Pucciarelli, 1991; Pucciarelli et al., 2003; Neves and Hubbe, 2005). However, recent papers (Powell and Neves, 1999; González-José et al., 2003) suggest that early remains from Brazil fall well within the range of variation of some modern Native American groups and that populations, or at least craniofacial patterns, survived and admixed until modern times. Considering the internal ranges of phenotypic variation observed across the New World, and taking into account that there are no *a priori* reasons to indicate the absence of admixture between any putative populations independently migrating from Asia, gene flow during the early phases of occupation should not be viewed as a rare event.

In our view, analyses of morphological variation focused on the reconstruction of historical processes can be improved by taking into account two facts. First, previous discussions about Asian-American affinities based on cranial data were centered on the use of somewhat simplistic morphological labels such as Paleoamerican, Amerindian, Mongoloid, and so forth. However, these labels tend to disregard the within-group variance/covariance matrix which, in combination with an accurate estimation of between-group variation, provides noticeably useful information about past microevolutionary events. In addition, classical categories such as Mongoloid, for instance, are ill-defined, and scholars greatly differ in its application to different local populations (Lahr, 1996).

Second, analyses of phenotypic variation across Asia and the New World were conducted using classical biometric methods, thus disregarding the power of geometric-morphometric analyses to study levels of within- and between-samples variation. Geometric morphometrics focus on the retention of geometric information throughout the analysis and provides efficient, statistically powerful tools that can readily relate abstract, multivariate results to the physical structure of the original specimens (Hennesy and Stringer, 2002; Slice 2007). Here, we suggest that proper approaches to cranial shape must consider its biological nature: shape is a geometric concept that is distributed on a multivariate and continuous spectrum of variation.

Considering this premise, the primary objective of this article is to reanalyze cranial variation in the Old and New Worlds using geometric morphometrics and interpret the results after multivariate statistical treatment rather than viewing these data as belonging to discrete categories. Thus, we are interested in a redefinition of the apportionment of variation in Asia and America and in the evaluation of the validity of the classical groups used in previous models. Our null hypothesis states that most New World samples are arranged between generalized and derived extremes of craniofacial variation, without important gaps between them. Since molecular markers indicate a moderate founder effect concomitant with the first entry into the Americas, a second null hypothesis is that there are also autochthonous patterns of craniofacial shape into the New World. Testing the latter hypothesis is of great interest since quantitative traits and uniparental markers have different effective population sizes and rates of mutations. Consequently, any discussion of the extent of the putative founder effect will be enhanced if the effect is tested on systems with different evolutionary timings.

A secondary objective of this paper is to attempt to reconcile the craniofacial results obtained here with the recent consensus achieved by geneticists to formulate an integrated model for the initial peopling of America two decades after Greenberg et al.'s (1986) synthesis.

MATERIALS AND METHODS
The sample

We analyzed twenty-three skull assemblages, including 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. Sex and age were estimated following diagnostic traits provided by Buikstra and Ubelaker (1994). When present, the pelvis was used to assign the specimen's sex. Otherwise, sex assignment was made based on cranial traits. Great care was exercised in the specimen selection to avoid sample bias, but the availability of material in museum collections did not allow us to gather data from age and sex-matched samples.

TABLE 1. Sample composition

Sample	Code	N (females/males)	N (total)
Late Pleistocene (Early) Old World	EOW	0/13	13
Herto, Ethiopia (160000–154000/160000–154000)*			
Cro Magnon, France (25000/25000)			
Keilor, Australia (12000 ± 100/13840)*			
Kow Swamp (13000–9000/15320–11170)*			
Liujiang Guangxi Zhuang, China (60000 ?/60000 ?)*			
Minatogawa, Japan (18000–16000/21280–19160)*			
Mladec, Czech Republic (31000/31000)			
Qafzeh 9, Israel (100000/100000)			
Skhul 5, Israel (90000/90000)			
Shakameyama, Japan (2300/2340)*			
Shosenzuka, Japan (13000–8000/15320–8985)*			
Upper Cave 1, Zhoukoudian, China (10175 ± 360/11910)			
Wadjak 1, Java (6500–10560/7415–12400)*			
Tchouktchi, Siberia	TCH	3/11	14
Buriats, Siberia	BUR	5/5	10
Ourga, Siberia	OUR	11/7	18
Ainu, Japan	AIN	3/7	10
Aborigines, Australia	AUS	16/20	36
North Paleoamericans	PAM	1/5	6
Chimalhuacán, Mexico (10500/12405)			
Kennewick Man, US (9300/10510)			
Metro Balderas, Mexico (9000/10195)			
Peñón III, Mexico (10755 ± 75/12810)			
Cueva del Tecolote, Mexico (10500/12590)			
Wizards Beach, US (9225/10405)*			
Eskimos, Greenland	ESK	28/18	46
California, USA	ACA	22/27	49
Baja California Sur, Mexico	BCS	11/12	23
Aztecs from Tlatelolco, Mexico	TLA	7/19	26
Paleoamericans from Brazil	LS	3/8	11
Capelinha (8860 ± 60/9830)*			
Sören Hansen 01 (7000–9000/7765–10175)			
Sören Hansen 02 (7000–9000/7765–10175)			
Sören Hansen 03 (7000–9000/7765–10175)			
Sören Hansen 04 (7000–9000/7765–10175)			
Sören Hansen 07 (7000–9000/7765–10175)			
Sören Hansen 09 (7000–9000/7765–10175)			
Sören Hansen 16 (7000–9000/7765–10175)			
Lapa Vermelha IV (11000–11500/12915–13300)			
Santana do Riacho III (8000–9500/8770–10690)*			
Santana do Riacho XXIII (8000–9500/8770–10690)*			
Mapure, Venezuela	MAP	17/21	38
Paltacalo, Ecuador	ECU	27/26	53
Ancon, Peru	PER	20/17	37
Aymará, Bolivia	BOL	6/12	18
Calama, Chile	CAL	12/12	24
Pampa Grande, Salta, Argentina	PG	16/9	25
Chaco, Argentina	CHA	2/8	10
Araucano, Argentina	ARA	26/17	43
North Patagonians, Argentina	NPA	9/9	18
Central Patagonians, Argentina	PAT	18/20	38
Fuegians, Chile and Argentina	FUE	7/3	10
Total			576

* Images not taken by the authors, collected from the literature.

All samples are modern (late Holocene) excepting LS, PAM, and EOW. Specimens integrating the Lagoa Santa (LS), north Paleoamerican (PAM), and late Pleistocene Old World (EOW) samples are presented separately to provide detailed information. Samples are arranged first for the Old World, and then for America, classified by ascending date and from north to south. Dates are given between parentheses as uncalibrated (radiocarbon) YBP/ calibrated (calendar) YBP. Code represents the grouping variable used in the PCA (see text for details).

Data acquisition

All skulls, excepting some ancient specimens (see Table 1), were photographed by the first author with a Sony Mavica MVC-CD350 camera (3.2 mega pixels of definition), according to the recommendations made by Zelditch et al. (2004). Prior to taking the photograph, 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 center of the field of view so that its image did not extend into the distorted region of the field. All images included a graded scale of 5 cm, as well as a label with the assigned sex, catalog number, entry number, and population assignation according to the Museum catalogue. Part of the photographic database used here had been previously analyzed in earlier papers

Fig. 1. Landmarks (solid points) and semilandmarks (open points) used in this study. pr: prosthion; ans: anterior nasal spine; zi: most inferior point on the zygomatic bone; ju: jugale; n: nasion; op: most posterior point on the orbital border; afm: anterior frontomalar; g: glabella; b: bregma; i: inion; po: porion.

(González-José et al., 2003; Neves et al., 2005; Martínez-Abadías et al., 2006). Landmarks and semilandmarks depicted in Figure 1 were digitized, scaled, and processed using TPSDig and TPSUtil (Rohlf, 2004a,b). Semilandmarks were placed along the contour of the cranial vault, and the TPSUtil routine was used to allow semilandmarks to slide so as to minimize bending energy (Rohlf, 2004b).

Geometric morphometrics

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; Hennessy and Stringer, 2002; Zelditch et al., 2004; Slice 2007). Geometric morphometric methods are based on the analysis of landmark configurations, each of them representing one individual. Original configurations were superimposed using the Generalized Procrustes Analysis (GPA) to remove the effects of translation, rotation, and scaling (Zelditch et al., 2004). After superimposition, shape was condensed in the aligned specimens, and size was expressed as the centroid size, which was computed as the square root of the summed distances between each landmark coordinate and the centroid (mean x, y, z, landmark for the configuration).

Thus, from this stage on and throughout the analysis, the differences observed between landmark configurations were only due to shape. From the superimposed configuration, a mean shape of individuals was obtained (the consensus shape configuration) and used as a reference. The shape of each individual was defined by Procrustes residuals, which are the deviations of landmarks relative to the consensus.

The next step was to apply the thin-plate spline (TPS) function (Bookstein, 1991) to obtain a new series of shape variables from the raw data, the partial warps, which allow the quantitative analysis of shape. The partial warp scores define the position of each individual in the shape space (Bookstein, 1991; Zelditch et al., 2004).

The partial warps represent nonaffine deformations and highlight changes at progressively smaller scales. To consider global affine transformations, the uniform component can also be included in the analyses.

Shape change can be visualized as deformation grid splines: two shapes are compared by analyzing the deformation patterns obtained from distortion of the first shape (the reference shape) onto the second one (the target shape). The Thin Plate Spline (Bookstein, 1991) interpolation function was applied on the adjusted landmark configurations using the TPSRelW software (Rohlf, 2003). The partial warps and uniform components were corrected for sex-related size differences using z-score standardization within each sex. This is a common method for removing sex-related size variation (Williams-Blangero and Blangero, 1989; Relethford, 1994; González-José et al., 2004).

The sex-standardized matrix of partial warps and uniform components was then subjected to a Principal Components Analysis (PCA) to ordinate and explore the main axis of the sample's morphological variation, as well as potential subgroups' formation. Craniofacial change across the first PCs was represented as grid deformations for the positive and negative values.

The Procrustes superimposition removes scale but not the allometric shape variation that is related to size. Previous analyses (Lahr and Wright, 1996; Rosas and Bastir, 2002) have revealed that, in modern humans, there is a very significant association between robusticity and cranial size, by which the larger the size of the skull, the greater the development of the cranial superstructures. Considering that robusticity traits were classically used to discuss levels of generalized (robust) versus derived (gracile) morphology, data analysis after the removal of allometric effects could be of great interest.

Statistical significance of the allometric component was tested by multivariate regression of shape variation (as evaluated from the partial warps and the uniform shape component) as a function of centroid size. To remove correlations between shape variables due to allometry, the residuals of original landmark coordinates on the natural logarithm of centroid size were calculated using the IMP_Standard6 software (Sheets, 2001). Afterwards, a second PCA was made on the residual shape variables to explore the nonallometric trends of morphological change. Both the shape and allometry-free shape PCA analyses were intended to test the null hypothesis that morphological variation in the New World can be interpreted as a continuum among extremes of phenotypic variation.

In addition, PC scores were used as input for a k-means cluster analysis. This method of clustering is very different from the Joining (Tree Clustering) and Two-way Joining methods (Hartigan, 1975). This procedure exactly obtains n clusters that are as distinct as possible. Computationally, this method can be viewed as a reverse analysis of variance (ANOVA). The routine will start with k random clusters and then move objects between these clusters to minimize variability within clusters and maximize variability between clusters (Hartigan, 1975). To explore different clustering patterning in our sample, we computed five analyses considering $k = 2$, $k = 3$, $k = 4$, $k = 5$, and $k = 6$ numbers of clusters in the sample. This procedure would test the second null hypothesis that morphological variants present in the New World are completely autochthonous and not observable in the Old World.
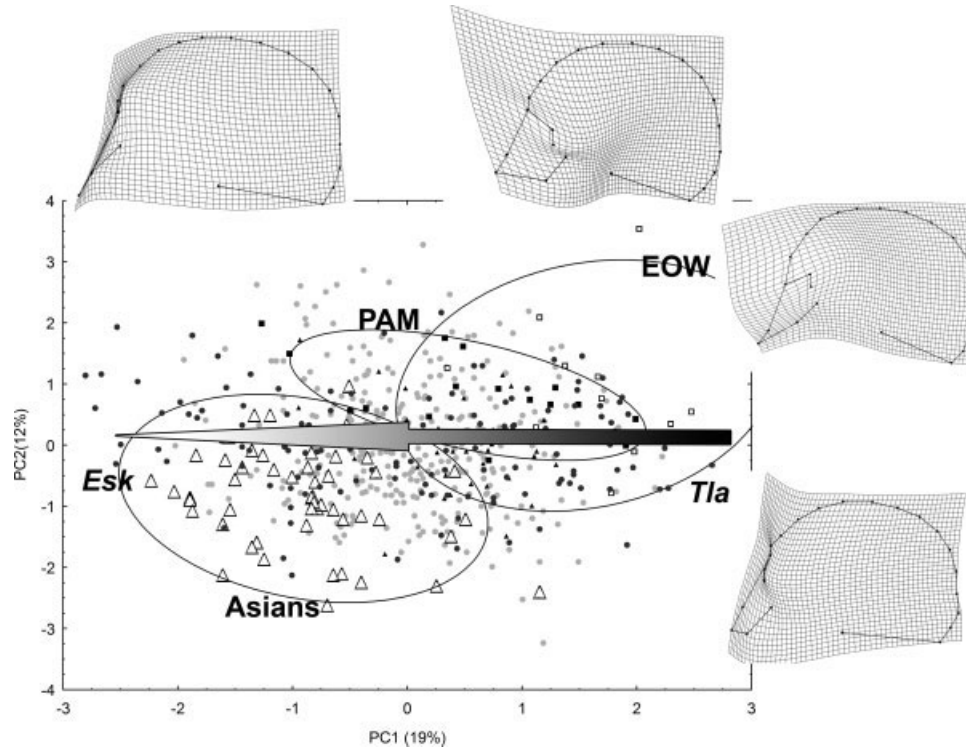
Fig. 2. Scatterplot of the two first Principal Components. Shape change across PCs is represented as grid deformations for the positive and negative values. Grids were exaggerated twice (2X). Ellipses of 95% confidence intervals are presented for the early Old World (EOW), Asian, and Paleoamerican (LS plus PAM) samples. Light gray circles: modern South Americans; dark gray circles: modern North Americans; solid triangles: Australians; open triangles: modern Asians; open squares: EOW; solid squares: LS+PAM. The grayscale vector represents the gradient of morphological change across America. Dark tones depict the generalized craniofacial pattern, gray tones the incipient and non synchronous presence of mongoloid traits, and light tones the derived morphology present in northeastern Asian and Eskimos. Italic labels mark the position of the two most divergent New World populations.

RESULTS

Results of the PCA and allometry-free PCA are presented in Figures 2 and 3, respectively. Both analyses show that whenever a wide range of geographic (from Australia to southern South America) and chronological (from late Pleistocene specimens to modern series) variation is considered, craniofacial phenotypes are not arranged into discrete units but rather in a continuous spectrum of samples. Scatterplots of the New World groups also show a rather continuous pattern. For example, ancient groups as the Lagoa Santa series, or modern groups from Baja California or Tlatelolcans, represent one extreme of variation that is closely associated to early Old World specimens; they are defined by low and projected faces, subnasal prognatism, 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, massive, anterior-projected and high zygomatics, and high noses. However, most of the New World samples fall well between both extremes (see Fig. 2).

Allometry was significant after 1000 permutations of the Wilk's λ parameter (Wilk's $\lambda = 0.820$; $p = 0.027$). Interestingly, the analysis made after removal of allometric effects show a very similar pattern of differences and affinities between groups (see Fig. 3). These allometry-controlled morphological differences are observable just after a 3–4× magnification (results not shown) and consist of a decreased variation of the glabellar and zygomatic regions in the allometry-controlled data, as compared to the nonallometry-controlled sample.

The K-means clustering results are presented in Figure 4. Australians and East Asians tend to form two major craniofacial clusters defined by specific color patterns (blue-green and orange-yellow, respectively). A striking result is the great differentiation observed among Early Old World (EOW) specimens, which, besides being a much reduced sample, occupy five out of the six clusters considered.

Regarding the clustering patterning in America, three main results are evident. First, there are no autochthonous clusters: all variants defining a cluster occur outside the continent. Second, sister groups of Native Americans, such as northeast Asians, present a homogeneous morphological pattern that is also observed among northern Native Americans (Eskimos) but whose prevalence decreases southward. In other words, there seems to be a circumarctic cluster (light blue) where north-east Asian and Eskimo skulls can be easily grouped. Third, the assignment of other Native American skulls into this cluster is a rare phenomenon, reflecting a particular geographical pattern of this phenotype, also observed in the white extreme of the vectors shown in Figures 2 and 3.
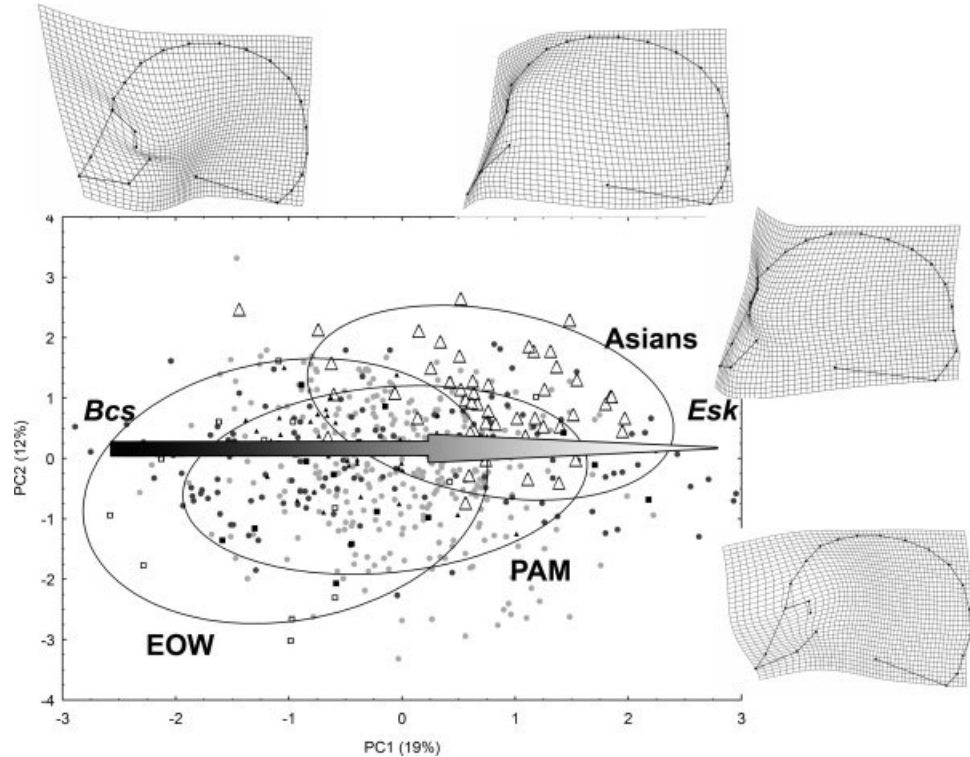
Fig. 3. Scatterplot of the two first principal components after removal of allometric effects. Details are the same as in Figure 2.

DISCUSSION

Our results indicate that we cannot reject the first null hypothesis of a continuum array of craniofacial variation in the New World. The generalized morphology defined by long neurocrania, projected and low faces, and relatively low and broad orbits is characteristic of early Old World specimens, Paleoamericans and some modern Native Americans. Conversely, derived traits such as extreme facial flatness in orbital and zygomatic projection, dolicocephaly, and high faces and noses are present among Asians and Eskimos. The evolutionary origin of this axis of phenotypic variation is still under debate and its discussion is beyond the scope of this paper. However, two main hypotheses for this pattern have been proposed. Somewhere in the range of distribution of the East Asian population, adaptation to a cold environment (Roseman, 2004; Harvati and Weaver, 2006) could have promoted the evolution of craniofacial traits from a generalized late Pleistocene ancestor to a set of derived, modern northeastern Asians. Harvati and Weaver (2006), however, emphasize that total cranial morphology does preserve a population history signal, as reflected by their results about correlations between neutral genetic distances and distances based on cranial morphology. Alternatively, incipient derived mongoloid traits could have been gradually inherited from a central Eurasian stock (Lahr, 1996), a connection that is also supported by the Y-chromosome distribution (Karafet et al., 1999; Santos et al., 1999; Bortolini et al., 2003; 2007). Since the two processes are not mutually exclusive, both adaptation and stochastic evolution could have promoted the expansion of vectors displayed in Figures 2 and 3 from the generalized to the derived extreme of craniofacial variation. If, as stated by Roseman (2004) and Harvati and Weaver (2006), the phenotype observed on circumarctic groups is the final result of directional selection after adaptation to cold climates, then a by-product of this event would generate a weakening of the continuous clinal pattern derived from larger differentiation of the adapted versus the nonadapted groups. Beyond the fact that Siberians and Eskimos tend to occupy an extreme of the phenotypic spectrum, the continuous nature of phenotypic variation among Native Americans is maintained even under acceptance of the scenario of directional selection acting upon the circumarctic groups.

Thorough descriptions of variation patterns among Native Americans and their role in the settlement of America were previously presented by Neves and Pucciarelli (1991), Lahr (1996), Jantz and Owsley (2001), González-José et al. (2003), Pucciarelli et al. (2003), Neves and Hubbe (2005). However, these studies tend to disregard the fact that most American groups occupy intermediate positions between the generalized and derived extremes. Consequently, the separation of samples into discrete categories represents a subjective assignment based on their affinities to any of the extremes of the morphological variation. This observation imply that labels such as "mongoloid," "proto-mongoloid," "premongoloid," and "Paleoamerican" should be used cautiously.

In addition, the evidence presented here indicates that the set of characters defining the range of New World phenotypic variation is already present in its putative ancestor, the late Pleistocene early members of our species, as well as in their two immediate sister groups: modern Australians and northeastern Asians. This is an important result concerning the settlement of the New World, since this EOW composite sample represents the best proxy to the ancestors of the first Americans and Asians. In this context, quantitative genetics theory
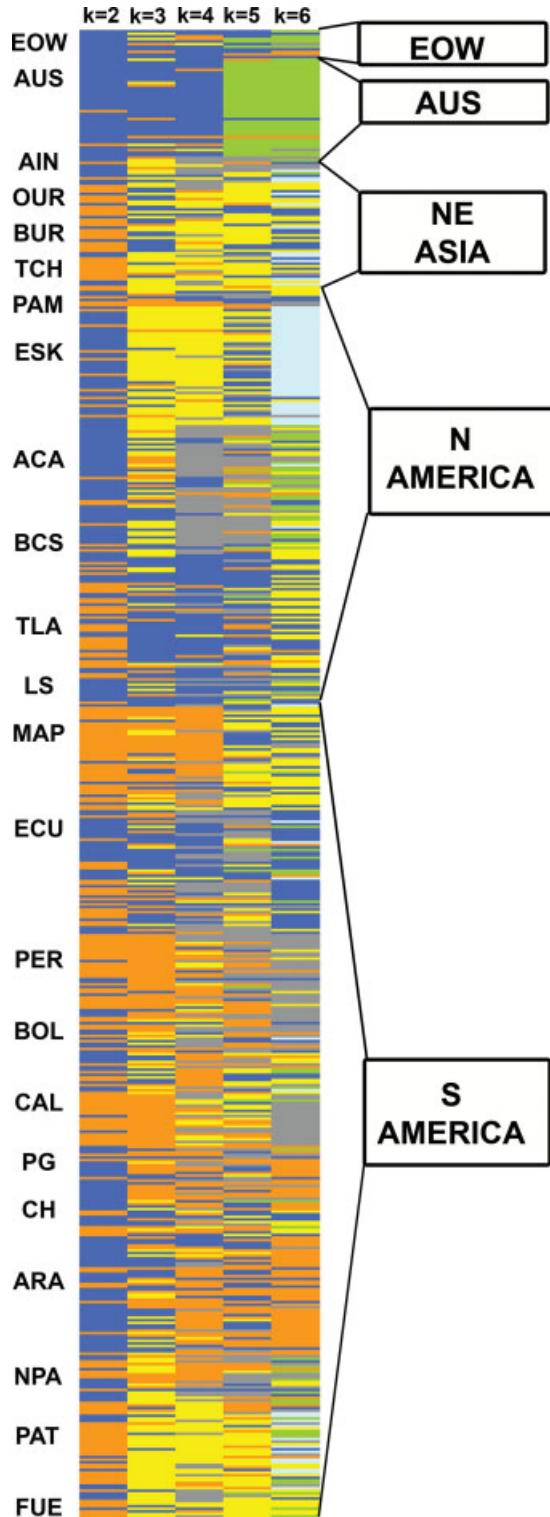
Fig. 4. Population structure as viewed from k-means cluster analysis. The number of clusters in a given plot is indicated by its k value. Individuals are represented as thin horizontal lines whose colors correspond to their membership in the phenotypic clusters. Top: Old World samples (from ancient to moderns, from north to south); center: North Americans (same criteria); bottom: South Americans (same criteria).

demonstrates that within-group variation in the ancestral population may be an important source of between-group diversity in the descendant groups (Lande, 1979). The lack of a founder effect signal on quantitative traits is an expected result given the polygenic and epigenetic nature of their genetic background, as well as the higher effective population size of this kind of characters in comparison to uniparental markers. It is also in close agreement with recent results from autosomal loci (Battilana et al., 2006; Fagundes et al., 2007).

In this context, the precursor of the single ancestral population, whose existence is suggested by mtDNA (Bonatto and Salzano, 1997a,b) or Y-chromosome markers (Tarazona-Santos and Santos, 2002), could well be a late Pleistocene Asian population inhabiting some place in northeast Asia and showing high degrees of craniofacial diversity. If craniofacial variation in the ancestral population was high enough to accommodate specimens with a mosaic characteristics or at least evolving from a generalized towards a specialized skull, then the demographic expansion of this population from north-East Asia is sufficient to explain, without serious contradictions, both molecular and craniofacial data in prehistoric America.

A remarkable result concerning the K-means clustering (but also evident in the PCA) is that one of the extremes of variation in the New World is observed among the North American Eskimos, who share their phenotype characteristics (in terms of assignation to a given cluster) mainly with modern northeast Asians. In agreement with recent molecular studies (Tamm et al., 2007; Wang et al., 2007), this shared pattern could indicate recent and pervasive gene flow among circumarctic groups. In summary, K-means cluster analysis allows a rejection of the second null hypothesis that autochthonous morphological phenotypes arose concomitantly with the settlement of the New World.

Toward a new interdisciplinary model for America's settlement

Over the last years, several settlement models have been presented to explain data that largely contrasted with the predictions of the Greenberg et al.(1986) model. Two examples can be given. The first is 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., 2008), which postulates that the molecular diversity of all modern Native Americans can be explained deriving from a single and early Beringian source. The second is the Two-Components model (Neves and Pucciarelli, 1991; Pucciarelli et al., 2003; Neves et al., 2003, 2005), which proposed that the presence of two differentiated craniofacial morphologies in America results from at least two chronologically separate waves with different Asian source populations. Although these alternative models can separately explain the molecular and morphological variability in the New World, they did not attempt to provide a more integrative view.

Starting with our geometric-morphometric analysis and the considerable bulk of new genetic data, we propose a model that considers both types of evidence. To facilitate its presentation, we have divided the process into four consecutive time frames, even though the studied event was a continuum process in space and time (see Fig. 5).
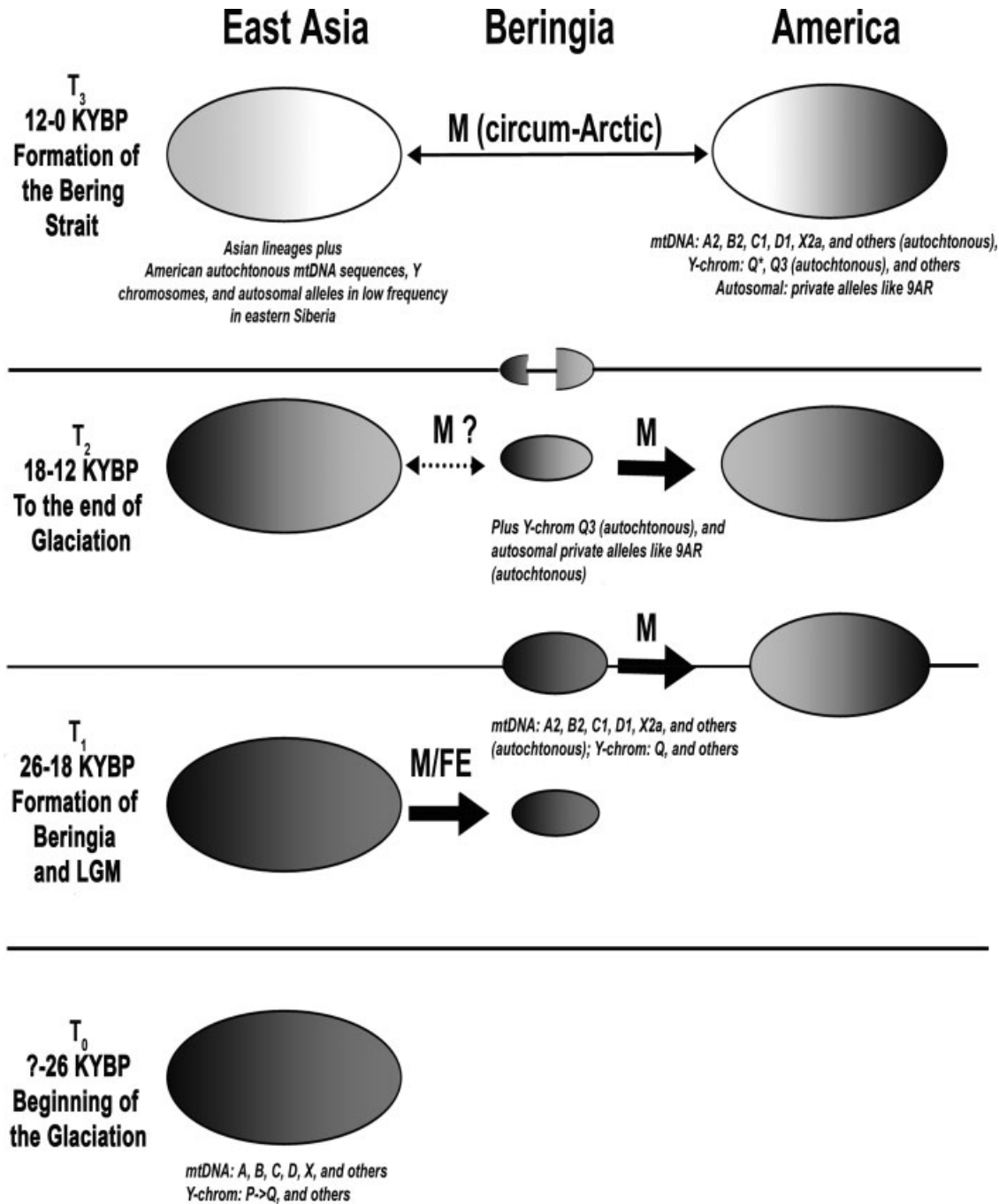
Fig. 5. Schematic representation of the model presented here. The settlement process was divided in four arbitrary periods to indicate evolutionary changes across time, given as calendar years. Ellipses represent populations at different times and regions. Size of the ellipses is an approximation to relative effective sizes. Grayscale gradient inside each ellipse represents variable amounts of specialization from generalized to derived morphology expressed as values across the vectors depicted in Figures 2 and 3. Main genetic characteristics for uniparental and other markers are depicted in the text-boxes. M: migration; FE: founder effect; KYBP: thousand years before present.

Our scenario begins in northeast Asia during the late Pleistocene, T_0, earlier than 26,000 YBP and at the beginning of the last glaciation. At that time, populations with an undifferentiated generalized and heterogeneous cranial morphology occupied the eastern rim of the Asian landmass, probably at the extreme of distribution of an initial expansion of *Homo sapiens* out of Africa. It is likely that the high within-group phenotypic diversity of this Asian stock (Brown, 1999; Cunningham and Wescott, 2002; Cunningham and Jantz, 2003) would not include in its range the complete expression of the traits later observed among northeast Asians. Indeed, these characteristics are absent from the fossil record of this period (Lahr, 1996; Brown, 1999; Cunningham and Wescott 2002; Cunningham and Jantz, 2003). Mitochondrial DNA haplogroups A–D and X and minor haplogroups as

well as the Y-chromosome lineage Q were likely present in the Asian population. Due to the relative scarcity of fossils for this period, little is known about the between-group variability and spatial distribution in East Asia at that time. This issue is important for identifying potential microregional population sources for the first Americans.

At T_1, between ~26,000 and ~18,000 YBP, a decrease in the sea level exposed a large land mass joining the northeast of Asia with northwest of America called Beringia, which during the last glacial maximum (LGM; 21,000 YBP) reached an area of about 1 million km^2. Human occupation of eastern Beringia, at the beginning of this period, is documented for the Yana site, which is dated to ~30,000 YBP (Pitulko et al., 2004). These populations would have occupied this region due to a rather mild climate (compared with its continental surroundings) and favored by the presence of game and fishing sites (Bobrowsky et al., 1990; Dixon, 2001). The Yukon Pleistocene fauna, for instance, included mammoth, horse, bison, camel and saiga, a grazing fauna that extended across Eurasia and Siberia. Furthermore, climate simulations suggest that winters were warmer and summers cooler than at present, and snow cover was thin or discontinuous (Schweger, 1997). Since the T_1 period includes the LGM summit, the occupation of open New World land masses (towards southern Alaska) would be impracticable due to the magnitude of North American glaciers.

Genetically, these populations would have many Asian lineages and, during this period, some of these lineages were likely lost by drift. Those that remained would have started their differentiation by means of accumulated mutations, defining the founder mtDNA and Y chromosome lineages, as well as autosomal private alleles such as 9RA at locus D9S1120 (Schroeder et al., 2006; Wang et al., 2007).

Taking into account that the evolution of skull morphology is strongly channeled by functional and development constraints (González-José et al., 2004; Lieberman et al., 2004) and that autosomal loci do not show a significant bottleneck effect (e.g., Battilana et al., 2006; Fagundes et al., 2007), the reduction of craniofacial variability at the beginning of this stage is expected to be not pronounced. Thus, the main morphological patterns observed at T_0 (high within-group variation, lack of extremely derived mongoloid traits) were probably conserved across T_1 and T_2.

In the next stage, T_2, between 18,000 YBP and the end of the Pleistocene period (~12,000 YBP), the Beringian landmass was being reduced due to sea level rise. Concomitantly, even when ancient Beringians were relatively isolated due to the presence of glaciers across North America, some coastal routes and/or ice-free pathways toward southern America were being opened (Bobrowsky et al., 1990; Dixon, 2001). Beringia's changing environment, combined with an increasing population size and relatively limited resources, may have stimulated further movements toward unexploited areas, southward the North American glaciers. Consequently, and as suggested by Dixon (2001), the initial human settlement of America may have begun in these dates along the southern margin of the Bering land bridge. According to this author, the Bering land bridge was important in this period since it provided an uninterrupted marine-intertidal environment that facilitated intercoastal navigation between the land masses (Dixon, 2001).

Mitochondrial DNA analyses indicate a significant population expansion at the beginning of this period (Fagundes et al., 2008). Thus, before the end of the LGM, a movement toward the south, likely along the Pacific coast, would give rise to the first Americans. With basically an undifferentiated or nonderived morphology toward the end of this stage, humans were certainly present all over the continent, as demonstrated by occupation of North American sites such as Fishbone Cave (Orr, 1956), Arlington Springs (Johnson et al., 2000), Meadowcroft Rock Shelter (Adovasio et al., 1980), and South American ones like Lagoa Santa (Neves et al., 1999b) and Monte Verde (Dillehay and Collins, 1988).

The T_2 Beringian population could well be represented by a particularly well-documented tradition recognizable in the archaeological record as the Nenana complex (Dixon, 2001; Goebel et al., 2003). This is the earliest recognized complex in eastern Beringia and is observable at several sites in interior Alaska. The earliest unequivocally dated cultural occupation in Beringia that can be placed into this time frame is the lowest component (unit 4c) of the Broken Mammoth site (Yesner, 2001) dated to around 14,000 YBP. The site is located in central Alaska and can be assigned to the early stages of the Nenana tradition. Contemporary to these dates, but on the Asian side, the confirmed cultural stratigraphy of the Ushki sites (Kamchatka, Russia), specifically a non-microblade industry overlain by a microblade industry, replicates the cultural sequence known for central Alaska (Goebel et al., 2003). This parallel sequence suggests the existence of a continuum across Beringia that enabled the existence of related cultures in both sides.

Whether the evolution of late Pleistocene generalized traits to modern derived ones was the result of adaptation, stochastic effects, or a combination of both processes, the craniofacial morphology of this period was characterized by high diversity in parallel with the absence of the set of traits defining an extreme derived phenotype as seen today only in Siberians and Aleut-Eskimos. In fact, the derived extreme of the vectors shown in Figures 2 and 3 was not present until very recent times (Lahr, 1996; Brown, 1999), at the period that we define as T_3. This sequence would explain why New World early skeletal remains (>10 KYBP) lack derived traits (Neves and Pucciarelli, 1991). In other words, the generalized and heterogeneous morphology seen among the first Americans, as well as the origin of incipient derived traits in the Late Pleistocene, would be the phenotypic parallel of a process that also involved genetic events like the fixation of autochthonous lineages and cultural shifts, such as the development of nonmicroblade tools in both sides of Beringia.

The T_3 period encompasses the Holocene and is distinguished by the origin of extreme northeast Asian morphology, its diffusion to America and concomitant *in situ* biological and cultural evolution. The extreme Siberian craniofacial pattern defined by facial flatness and zygomatic projection and size first arose in Asia at least 7,500 YBP (Brown, 1999), and its presence in northern North America could be due to low but constant gene flow among circumarctic populations. 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). For instance, microblade and burin industries appear synchronously in the

archeological records at Ushki (component 6) and in Alaska (the Denali complex), shortly after 12,500 YBP (Hamilton and Goebel, 1999). To Goebel et al. (2003), this sudden and significant technology reorganization could likely represent modern migrations of northeastern Asians into the region.

The evolution and diffusion of an extremely derived north-east Asian phenotype, the high heterogeneity of founder groups, and the beginning of *in situ* New World evolution shaped by migration and genetic drift explains the entire pattern of past and present Native American variation. Most modern populations can be shown to have a mosaic of generalized-derived traits, while a few of them (Aleut-Eskimos) display the derived extreme also present in northeast Asia, and others present a rather generalized, ancestral morphology (Pericu, Aztecs, and Paleoamericans). In summary, this model is a parsimonious explanation that reconciles genetic evidence for a single major origin and high craniofacial heterogeneity of Native Americans.

CONCLUSIONS

As presented in our synthetic model, the main assertions of previous models may not be in serious contradiction with each other but collectively contribute to depict a common picture. In this context, the Two-Components model is viewed here as highlighting the extreme phenotypic values observable both in Asia and the New World, while their origin is explained by microevolutionary agents such as drift, gene flow, and maybe directional selection. The significant amount of intermediate morphological variation, rather than these extreme values, is of crucial importance in our synthesis. Likewise, the Out of Beringia or Single Wave model is here reinterpreted in a more flexible way, with the proposal of a more continuous pattern of contacts among circumarctic groups.

We close by emphasizing the critical and dynamic role that Beringia played in several steps of our model: it was the homeland of Native Americans, and the initial setting for some of the most important evolutionary processes leading to at least an important part of their present-day biological and cultural diversity.

ACKNOWLEDGMENTS

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