Phenotypic Evolution of Human Craniofacial Morphology After Admixture: A Geometric Morphometrics Approach

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ABSTRACT An evolutionary, diachronic approach to the phenotypic craniofacial pattern arisen in a human population after high levels of admixture and gene flow was achieved by means of geometric morphometrics. Admixture has long been studied after molecular data. Nevertheless, few efforts have been made to explain the morphological outcome in human craniofacial samples. The Spanish-Amerindian contact can be considered a good scenario for such an analysis. Here we present a comparative analysis of craniofacial shape changes observed between two putative ancestor groups, Spanish and precontact Aztecs, and two diachronic admixed groups, corresponding to early and late colonial periods from the Mexico's Central Valley. Quantitative shape comparisons of Amerindian, Spanish, and admixed groups were used to test the expectations of quantitative genetics for admixture events. In its simplest form, this prediction states that an admixed group will present phenotypic values falling between those of both parental groups. Results show that, in general terms, although the human skull is a complex, integrated structure, the craniofacial morphology observed fits the theoretical expectations of quantitative genetics. Thus, it is predictive of population structure and history. In fact, results obtained after the craniofacial analysis are in accordance with previous molecular and historical interpretations, providing evidence that admixture is a main microevolutionary agent influencing modern Mexican gene pool. However, expectations are not straightforward when moderate shape changes are considered. Deviations detected at localized structures, such as the upper and lower face, highlight the evolution of a craniofacial pattern exclusively inherent to the admixed groups, indicating that quantitative characters might respond to admixture in a complicated, nondirectional way. Am J Phys Anthropol 000:000–000, 2006.

The mechanisms by which the shape of a complex structure, such as the human skull, results from the integration of morphogenetic rules, plastic responses, and evolutionary forces are not well-established (Lieberman et al., 2000a). One way to further understand the phenotypic expression of cranial morphological variation is to explore how the skull responds to the predictions of classical quantitative genetics. The detection of deviations from such expectations is crucial, since several models of population genetics are available for quantitative trait data (Konigsberg, 2000; Relethford, 2002; Sparks and Jantz, 2002; González-José et al., 2004). In this perspective, the Spanish-Amerindian contact can be considered a good scenario to test the effects of a given microevolutionary agent, such as admixture, on the phenotypic evolution of craniofacial human morphology.

Classical quantitative genetics theory predicts that whatever model of structure is used, gene flow has the effect of homogenizing the genetic composition. If gene flow is the only factor operating, then any two populations will converge to the same allele frequency, generally an average of the initial gene frequencies (Futuyma, 1986). Gene flow has the same effects on quantitative traits as it has on single-locus genes: migration reduces differences between groups, but increases variances within demes (Konigsberg, 2000). Therefore, according to quantitative genetics, an admixed group will present phenotypic values falling between those of both parental groups, in a position determined by the relative contribution of each parental group. For instance, Liu et al. (1996) demonstrated that the morphology of the posterior

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lobe of the male genital arch in F1 hybrids resulting from crosses of *Drosophila mauritania* and *D. simulans* was placed directly between the two parental types.

However, when the phenotype analyzed consists of complex structures, such as the vertebrate skull, the test for this prediction is not straightforward. Several factors such as morphological integration (Olson and Miller, 1958; Marroig and Cheverud, 2001; Bookstein et al., 2003), developmental and functional constraints (Lieberman, 1997; Lieberman et al., 2000a, 2004; Pucciarelli et al., 2000), and different levels of plasticity (Kiliaridis, 1995; Wood and Lieberman, 2001; Giesen et al., 2003) are thought to interact through ontogeny until the expression of adult morphology is achieved. As a result of morphological integration, it is expected that functionally and developmentally related characters will be inherited together. Environment also plays an important integrative role, since selection favors functional related traits, which evolve as a single coordinated unit (Cheverud, 1995). The complexity of such mechanisms makes it difficult to explore the basic phenotypic output expected under the effect of a given microevolutionary agent, like gene flow (Chakraborty, 1990). However, interesting clues can be obtained by studying different localized skull regions in order to verify which structures fit well the prediction of quantitative genetics, and which ones significantly depart from it.

While admixture on human populations has long been studied by means of serological and molecular markers (Wijsman and Cavalli-Sforza, 1984; Chakraborty, 1986), few efforts have been devoted to test levels of admixture on quantitative traits. Thus, the craniofacial expression resulting from such admixture events remains largely unexplored. Jantz (1973) and Key and Jantz (1981) attempted to explore gene flow between human populations after craniofacial data. By means of multivariate analysis, it was shown that temporal change in Arikara crania could be explained by admixture between Mandan and white populations with the Arikara population. More recently, Ross et al. (2004) analyzed the among-sample morphological variation of modern and precontact Cubans, Spanish, and Africans by means of three-dimensional (3D) geometric morphometrics in order to develop forensic identification criteria for hybrid Hispanic populations. These works suggest that multivariate and geometric morphometrics approaches to craniofacial variation enable the reconstruction of population microevolutionary and genetic underlying patterns, even when these may be obscured by the phenotypic nature of craniofacial traits.

Furthermore, a systematic approach to admixture on quantitative traits may yield interesting results: the gene flow process can be reconstructed from its beginning to its current stage. This can be achieved by studying diachronic series of the admixed population and by comparing if (given two morphologically divergent ancestors) both global and localized craniofacial shapes of the admixed groups present the morphological outcome expected under theoretical quantitative genetics. To carry out such a comparative analysis, samples should achieve some particularities. First, they should come from populations with high effective population sizes, in order to diminish the effect of genetic drift. Second, parental groups should be morphologically divergent enough to guarantee the detection of shape changes. Finally, both parental and admixed groups should share similar lifestyles and levels of mechanical stress.

The Spanish colonization of Mexico's Central Valley represents a scenario which largely fulfills the above requirements. Here we present a comparative geometric morphometric analysis of craniofacial shape changes observed between two putative ancestral groups, Spanish and precontact Aztecs, and two diachronic admixed groups, corresponding to early and late colonial periods. Both ancestral populations were large in size, diverged genetically and morphologically, and practiced extensive farming. An additional advantage is that Mexican precontact and colonial remains are abundant and well-dated. Finally, the ultimate result of the admixture process can be derived from molecular admixture rates estimated for the modern population (Lisker et al., 1996; Cerda-Flores et al., 2002). The main goal here is to carry out a systematic, diachronic, quantitative approach to evaluate if temporal craniofacial variation in Mexican and Spanish samples can be explained by admixture and gene flow among them. We test the hypothesis that the craniofacial phenotype reflects the admixture process in Mexico's Central Valley in a congruent way with molecular and historical studies. Moreover, we test whether the picture given by the craniofacial phenotype remains the same when localized structures, rather than the entire skull, are analyzed. The null hypothesis expects that craniofacial data will fit the predictions of classical quantitative genetics, and hence the morphology of the admixed groups will fall between the range of morphological variation shown by the ancestor samples. If so, admixture levels inferred after morphometric estimates will be in accordance with the final step of the gene flow process. Given the continued gene flow process during the three centuries of Spanish colonization, the early colonial admixed group is thus expected to show more affinity to the Amerindian ancestor, whereas the late colonial group is expected to show more affinity to the Spanish one. Moreover, the late colonial is expected to reflect admixture levels inferred after genetic markers in present populations from Mexico's Central Valley. Conversely, if deviations from the expected pattern are detected when analyzing either global skull morphology or localized shape structures, the null hypothesis will be rejected. This would show that craniofacial traits do not respond directly to external stimuli and microevolutionary agents such as gene flow.

HISTORICAL AND DEMOGRAPHICAL BACKGROUND

Spanish colonization of the Americas led to high levels of admixture and gene flow. As stated by Sans (2000), this microevolutionary process so deeply affected Amerindian population structure that the Latin American continent can be considered a natural experiment for admixture studies. As a result of colonization, diverse populations such as Amerindians, Europeans (mainly Spanish and Portuguese), and Africans (introduced by conquerors as slaves) entered into contact and mixed among themselves. To study admixture in Latin America, a trihybrid admixture model is generally considered; however, the contribution of each population to the Amerindian gene pool differs among regions, depending on biological and historical facts.

In Mexico, since Spanish colonization in 1521 by Hernán Cortés, admixture has been one of the main mechanisms contributing to the evolution of its population structure and history. Almost three centuries of

Spanish colonization brought many social, cultural, and political consequences to Amerindian societies, as well as biological. Initially, the Spanish conquest brought indigenous populations to demographic collapse (Márquez Morfín, 1993), with high mortality rates due to war, epidemics, hunger, and overexploitation. Later, it gave rise to a new admixed group, the so-called *Mestizos*, and a strong hierarchical system of *castas* that limited exogamy among individuals belonging to different social groups. In general terms, these events summarize the main biodemographical impact of Spanish colonization on the population structure of Mexico. However, the picture is quite diverse throughout the Mexican territory. In the present study, we focused on the Mexican Central Valley, where we derived our samples.

Spanish colonization of Mexico can be reconstructed thanks to the preservation of many historic records from the colonial period. Unfortunately, this is not the case for many Prehispanic documents. Historic-demographic studies in Mexico are abundant and are based on ecclesiastic, fiscal, and civil records. Nevertheless, the reliability of such sources of information has been challenged, and figures provided by different authors fluctuate widely.

The Spanish colonization started in 1519 with the arrival on the coast of Veracruz of 633 individuals coming from the Antilles islands and commanded by Cortés. After several exploratory expeditions, the initial group grew and reached 2,329 individuals (Velasco, 1993). In 1521, the Spaniards conquered the capital of the Aztec Empire, the *Great Tenochtitlán*, and declared Mexico part of the Spanish Empire, naming it Nueva España. In order to consolidate the conquered territories, the Spanish authorities promoted the migration and settlement of European population. According to the *Catálogo de Pasajeros a Indias*, between 1509–1559, 15,000 people travelled to the Americas; other documents raise this figure up to 40,000 or 200,000. However, the migration of women and of entire families was not very successful. The first colonists in Mexico came mainly from the Iberian Peninsula, with Andalusia (33.3%), Castille (28.1%), and Extremadura (17.3%) being the most important sources of migration (Sánchez Albornoz, 1977). Díez de la Calle (1932) pointed out that by 1646, 13,780 Spanish were settled in 18 cities of Nueva España, the majority living in Mexico City (58%), followed by Puebla (7.3%) and Atlixco (7.3%).

The starting point of the colonial period saw a drastic decrease of the Amerindian population, the causes of which were war, the social, economical, and cultural collapse of indigenous societies, and epidemics brought by European colonists. It was reported that during the 16th century, several generalized epidemics (1520–1521, smallpox; 1545–1548, *cocolitzi*, which was the *Nahuatl* term that Amerindians used to refer to any epidemic disease, e.g., measles or mumps; and 1576, *matlazáhuatl* or exantemic typhus), as well as bad harvests, provoked severe demographic crises in Mexican populations (Márquez Morfín, 1993). The lowest level of Amerindian population size was reached somewhere between the end of the 16th century and beginning of the 17th century, when the population started to recover, and the number of colonists increased. By that time, admixture was one of the main processes responsible for this population increase. Demographic growth began during the first half of the 17th century and was accelerated during the second half. Nevertheless, during the last decade of the 17th century, the growth rate slowed down and reached stability during the 18th century (Márquez Morfín, 1993).

Gibson (1961) reported that the Amerindian population size in the Central Valley of Mexico at the beginning of the Spanish conquest was about 1.5 million inhabitants, which was reduced to 325,000 effectives in 1570, and to 70,000 by the middle of the 17th century. Afterwards, the population increased and regained its initial size by 1800. The growth rate in Mexico during the period 1793–1810, fluctuates between 1.9–2.7. Moreover, Mexico was one of most populated provinces in the colonial period, along with Guanajuato, Puebla, Oaxaca, and Michoacán. In 1793, the number of inhabitants was 1,162,856; in 1803, 1,511,900 (with a population density of 12.9 inhabitants per km^2); and by 1810, 1,591,844 (with a population density of 13.6 per km^2).

Several circumstances favored the demographic recovery of indigenous Mexican populations: immunological adaptation to epidemic diseases, improvements in quality of life such as better nourishment and hygienic conditions, reduction of overexploitation, and increased legal protection of the indigenous population. But admixture also had a very important role, mainly due to the fact that the colonizing population was in strong disequilibrium between the sexes and needed to reproduce. Aguirre Beltrán (1972) pointed out that by 1793, the European colonists were mainly adult males: almost 75% were between 29–40 years old, and only 1.5% were females. Conversely, the Amerindians were a predominantly young, growing population: nearly 50% were under 20 years old. This great disproportion necessarily compelled the colonizing populations to mix with the Amerindians, because the European population established in America did not have the capability to self-reproduce its population (Aguirre Beltrán, 1972). Aware of this need, the Spanish authorities encouraged marriages at earlier ages, tolerated free unions and secondary nuptials, and allowed Amerindians to marry with people belonging to other social groups, thus promoting admixture.

Throughout the colonial period, the admixture process was consolidated, and the admixed groups showed an accelerated natural growth. By 1570, the admixed groups represented only 0.5% of the total population, but by 1810 represented as much as 39.5%. The number of intermarriages was great and produced many admixture classifications, depending on the origin, color, and social group or *casta* (Velasco, 1993). The great majority of *Mestizos* resulted from Spanish and Amerindian admixture, whereas the admixture between Amerindians and Africans was not as frequent. The magnitude and features of the admixture process differed among regions. García Martínez (1990) reported that in Mexico City, the ethnic composition of the population in 1810 was made up of 269,416 Spanish and *Criollos*, who were the descendants of the Spanish who were born in *Nueva España*, (17%); 1,052,862 Indians (66.3%); and 265,883 individuals belonging to another *castas* (16.7%). However, this author found that the Indian group also included high percentages of *Mulatos* (resulted from the admixture between Spanish and Africans), *Mestizos* (Spanish and Amerindians), and *Pardos* (this later group resulted from admixture between an Indian and a *Mulato*). In Tlaxcala, the percentage of Indians was higher, at 72.4%. The number of *Mulatos* in this region was reported to be very low.

TABLE 1. Archaeological details, sex composition, and sample sizes

Sample	Code	Region	Dating	n (female)	n (male)	n (total)
Spanish ancestor[1]	SA	Madrid	19th century	22	22	44
Amerindian ancestor[2]	AA	Tlatelolco, Mexico D.F.	Late Postclassic	15	15	30
Santa María Texcalac[2]	SMT	Tlaxcala	17th century	4	4	8
Hospital San Juan de Dios[2]	HSJ	Mexico D.F.	19th century	12	12	24
Total				53	53	106

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ADMIXTURE STUDIES IN MEXICO

Several studies based on anthropometrics (Faulhaber, 1955), dental traits (Baume and Crawford, 1978), and dermatoglyphics (Serrano, 1982) are the first attempts in the literature to evaluate admixture in Mexican populations. These studies were mainly based on the computation of biological distances between admixed and nonadmixed Mexican populations; they then assessed if there were statistically significant differences among these groups. Thereafter, admixture studies estimated the influence of Amerindians and Spanish in the admixed Mexican groups. Domínguez Olivier (1984) compared the dermatoglyphic patterns of three *Mestizo* socially heterogeneous groups from Mexico City with Indigenous and Spanish populations, and concluded that the admixed groups were at an intermediate point between the Indigenous and the Spanish. Furthermore, it was found that differences among admixed groups could be explained by socioeconomic factors. López Alonso (1990) compared dermo-papillar traits of an admixed population of Mexico City with Amerindian and European populations, and showed low Amerindian, but high European contributions to the admixed group. Finally, admixture studies based on genetic and molecular markers (blood groups, proteins, the Y-chromosome, autosomic, and mitochondrial DNA) are abundant. Here, only those studies dealing with the Central Valley of Mexico populations will be referenced, but for a general revision, see Lisker et al. (1996). Admixture figures based on classic genetic markers are provided for Mexico City and for Tlaxcala. In Mexico City, several studies were carried out, but results differed because of sample choice. Tiburcio et al. (1978) reported 27.6% Amerindian, 70.8% European, and 1.4% African contributions, whereas Lisker et al. (1986) showed a much lower contribution of European populations, with 56.22% Amerindian, 40.85% European, and 2.93% African. Cerda-Flores et al. (2002) computed admixture contributions, analyzing the frequencies of molecular markers D1S80 and HLA-DQA1, and found a European contribution of 50.03%, an Amerindian contribution of 49.03%, and a very low African contribution, of just 0.94%. For Tlaxcala, the admixture figures provided by Crawford and Devor (1980) showed a 70% Amerindian contribution, 22% European, and 8% African.

MATERIALS AND METHODS

Four skeletal samples, including two ancestral populations (one Spanish and one precontact Amerindian) and two postcontact populations from Mexico's Central Valley were analyzed (Table 1). The total sample includes 106 complete adult skulls of both sexes. Male and female individuals are represented by equal numbers, pooled in sex-balanced samples in order to carry out statistical analyses. Data were collected from digitized images in the form of two-dimensional (2D) coordinates of craniofacial landmarks, using the tpsDig program (Rohlf, 1998a). Nineteen landmarks, covering both facial and neurocranial regions, were located on the lateral profile of each skull, most representing standard osteological points (Table 2 and Fig. 1). Some sample sizes are quite small even if males and females are pooled, because the number of variables is greater than the number of individuals. This is especially the case in the early colonial sample (SMT). Therefore, we replicated all analyses without including it and by considering fewer landmarks, to confirm the suitability and validity of the results presented here.

TABLE 2. Digitized landmarks[1]

No.	Landmark	Code	Description
1	Prosthion	pr	
2	Subspinale	ss	
3	Nasospinale	ns	
4	Nasion	n	
5	Glabella	g	
6	Supraglabellare	sg	
7	Metopion	m	
8	Bregma	b	
9	Vertex	v	
10	Point A	pA	Midline point of greatest elevation between bregma and lambda
11	Lambda	l	
12	Opisthocranion	op	
13	Inion	i	
14	Porion	po	
15	Point B	pB	Most inferoposterior point on zygotemporale suture
16	Jugale	ju	
17	Zygomaxillare	zy	
18	Orbitale	or	
19	Frontomalare orbitale	fmo	

[1] See Bräuer (1988) for landmark definitions, except where described further.

The Spanish ancestor (SA) was represented by a sample of Spanish individuals from Madrid. Taking into account that Spanish colonists came from quite diverse regions, the Madrid sample was chosen because it is a good representative of the Spanish population. Moreover, several works on spatial and temporal cranial variation in the Iberian Peninsula (Garralda and Mesa, 1984; Lalueza Fox et al., 1996) have shown that, despite temporal and geographical divergence, the morphological homogeneity among Iberian populations is high.

The Amerindian ancestor (AA) was represented by a Late Postclassic (1350–1400) sample from Mexico D.F. Tlatelolco, jointly with Tenochtitlán, was one of the main

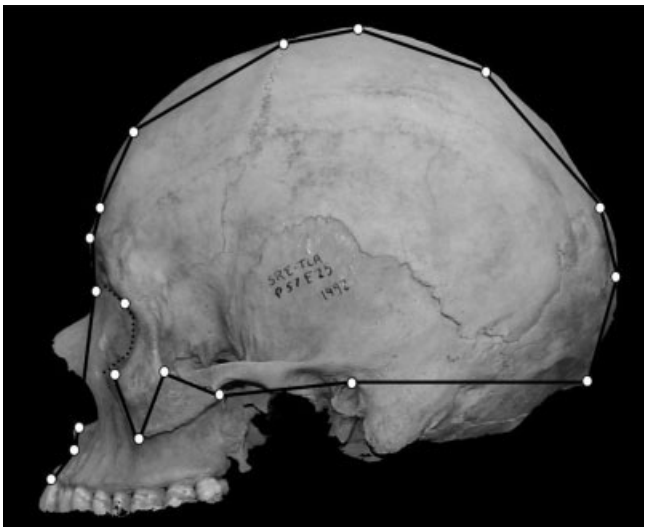

Fig. 1. Craniofacial landmarks shown on lateral view of an Amerindian skull from Tlatelolco. Dots represent landmarks; straight lines between landmarks are links used for convenience in visualization.

cities of the Aztec Empire. The materials excavated from this archaeological site present excellent skeletal preservation and are abundant. Thus, only those individuals not presenting artificial deformations were included in the analyses. These materials are preserved at the Dirección de Antropología Física (Instituto Nacional de Antropología e Historia, Mexico City).

Finally, two other samples from Mexico's Central Valley are representative of early and late colonial periods. The Santa María Texcalac sample (SMT) comes from the excavation of burials of a 17th–18th century Franciscan convent in Tlaxcala. A colonial census of Tlaxcala showed that the population was principally made up of Spanish and Amerindians initially (1556–1557), but by 1779 the *Mestizo* group had become increasingly prominent. Meanwhile, the Hospital San Juan de Dios sample (HSJ) was recovered from the excavation of a 19th century hospital from Mexico City, and it is assumed to be representative of the urban population of that period.

Given the samples used in the present study, a dihybrid admixture model is considered to evaluate admixture at populations from Mexico's Central Valley. Thus, only the European and Amerindian contributions will be taken into consideration. Historic, demographic, and genetic evidence indicates that the African contribution to this region is low, precluding the use of an African sample. This would not be the case in other Mexican regions, especially coastal, where the African contribution was found to be high (Lisker et al., 1996).

Geometric morphometric analysis

The captured craniofacial shapes were processed by means of geometric morphometrics, a useful approach for the quantitative characterization, analysis, and comparison of biological form (Bookstein, 1991; Marcus et al., 1996; Dryden and Mardia, 1998; Lele and Richtsmeier, 2001).

Geometric morphometric methods are based on the analysis of landmark configurations, each of them representing one individual. These data are then processed by means of statistical shape analyses. However, before carrying out analyses, the sample was tested to fit several assumptions underlying geometric morphometrics. First, isotropy of the data is required: landmarks are expected to be symmetrically distributed around the Procrustes mean. A visual inspection of 2D scatterplots around the landmark centroids is not sufficient (Lele and Richtsmeier, 2001), so following Dryden and Mardia (1998), we tested if our data followed the isotropic model by computing a principal components analysis on the raw data matrices of the four samples, expecting the first principal components to account for low levels of variability. Next, a test was done to see if the projection onto a tangent space of the fitted coordinate configurations lying in Kendall's shape space was a good approximation for the data. Thus, a correlation between Procrustes and Euclidean distances was computed, using the tpsSmall program (Rohlf, 1998b).

Once these tests were performed, the first step was to compute a generalized Procrustes superimposition (Goodall, 1991; Rohlf and Slice, 1990), in which landmark configurations are translated, scaled, and rotated according to a least-squares criterion until the distances between homologous landmarks are minimized. Thus, from this stage and throughout the analysis, differences observed between landmark configurations are only due to shape (Rohlf, 1990; Rohlf and Marcus, 1993). From the superimposed configuration, a mean shape of individuals is obtained (the "consensus" shape configuration) and used as a reference. The shape of each individual is defined by Procrustes residuals, which are the deviations of landmarks relative to the consensus.

The next step was to apply the thin-plate splines (TPS) function (Bookstein, 1991) in order to obtain a new series of 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, 1996; Rohlf, 1998c), and they are collected in the so-called "weight matrix." The partial warps represent nonaffine deformations, and highlight changes at progressively smaller scales. In order to consider global affine transformations, the uniform component can be included in the weight matrix. Thus, the weight matrix has as many columns as partial warps (plus two columns if the uniform component is considered), and contains as many rows as individuals. 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 deformation requires bending energy, whose computation leads to partial warp scores. Thanks to the properties of the TPS interpolation function (Bookstein, 1991), these new variables can be projected into a linear tangent space and analyzed by means of traditional multivariate techniques. The weight matrix was obtained using the tpsRelw program (Rohlf, 2003a).

A canonical variates analysis (CVA) using discriminant function was performed to maximize separation among samples and to explore shape variation. The CVA was performed on the weight matrix using the Statistica 6.0 software package (Statsoft, Inc.). This test derives some discriminant functions, resulting from an optimal combination of variables (in this case, the partial warps), so that the first one provides the most overall discrimination between groups; the second provides second most,

and so on. Moreover, the functions are independent or orthogonal, i.e., their contributions to discrimination between groups will not overlap (Manly, 1994). Computationally, a canonical correlation analysis is computed in order to determine the successive functions and canonical roots, where the term "root" refers to the eigenvalues that are associated with the respective canonical function. The maximum number of functions that is computed is equal to the number of groups minus one. In summary, this analysis examines relationships between and within groups, and reflects their patterns and degree of morphological variation (Dryden and Mardia, 1998). To visualize shape changes, grid deformations that could be associated with either high or low values of each canonical variate were generated using the tpsRegr program (Rohlf, 2003b). Thin-plate splines were obtained after computing the regression of partial warps onto the three canonical variates. This is a useful method for detection of influential landmarks and assignation of specific global and localized shape changes to each sample.

Mahalanobis distances were computed using the Statistica 6.0 package in order to assess the degree of differentiation among groups and its statistical significance. The *P*-value obtained was adjusted for lack of independence, using the Bonferroni method: the total comparisons were $c = k(k - 1)/2$ (where k is the number of populations), and the significance level 0.05 was divided by c to guarantee real significance.

Complementary to TPS, a Euclidean distance matrix analysis (EDMA) was computed using the WinEdma program (Cole, 2002) in order to further compare craniofacial shapes. EDMA (Lele and Richtsmeier, 1995, 2001; Richtsmeier et al., 2002) is a coordinate-system-free approach that is invariant to shape orientation (Lele and Richtsmeier, 2001). While EDMA methods also use landmark coordinates as raw data, the form of each individual is here represented by the form matrix (FM), i.e., the matrix of Euclidean distances between all possible unique landmark pairs (Lele and Richtsmeier, 2001). The form matrix (or FM(A) for object A) is an equivalent representation of the landmark coordinate data that is invariant to the nuisance parameters of translation, rotation, and reflection (Lele and Richtsmeier, 2001). The mean shape matrices for each sample were obtained after standardizing the mean form matrices of each sample by a scaling factor, the geometric mean. The scaled interlandmark differences found among populations were then used to explore localized skull shape changes (Lele and Richtsmeier, 1995, 2001; Richtsmeier et al., 2002). Lele and Cole (1996) described a procedure for testing for significant differences in shape and size, based on computation of the z-statistic. The statistical significance of localized shape differences was tested using a Monte Carlo approach, a parametric bootstrap procedure to calculate the 100 $(1 - \alpha)\%$ confidence interval for each size-corrected linear distance (Lele and Cole, 1996; Lele and Richtsmeier, 2001). Confidence intervals were obtained after 999 iterations, and $\alpha = 0.1$. According to EDMA-II testing, a particular interlandmark distance is considered to be equal in two given samples if the resulting interval contains the value zero. Otherwise, the equality null hypothesis is rejected, and it is assumed that a significant shape difference exists at the α level in that specific region (Lele and Cole, 1996).

An exhaustive comparative analysis of the sorted elements of the shape difference matrices was performed to test predictions of quantitative genetics for admixed populations, by detecting if interlandmark distances between the colonial and parental groups were intermediate to the interlandmark distance separating both ancestors. The distances that did not accomplish this expected pattern were considered as deviations from the quantitative genetics theory.

Shape changes were subdivided into major and moderate, and were handled separately. We refer to major shape changes as those scaled linear distances which were $\geq$10% longer or shorter in the reference than in the target mean shapes, whereas those distances $\geq$5 or $<$10% longer or shorter in either of the two shapes were considered moderate shape changes. Finally, major and moderate interlandmark distances were discussed in terms of their topographical localization, in order to explore potential effects of morphological integration and developmental constraints. Following previous works (Cheverud, 1995; Marroig and Cheverud, 2001), interlandmark distances were assigned to a particular region in the skull: either to the cranial vault and the orbit, which are supposed to follow a neural growth pattern, so being formed from paraxial mesoderm-derived cells; or to the face, a structure assumed to be the result of a somatic growth pattern and derived from neural crest cells.

Major shape changes were graphically represented by means of all possible pairwise comparison schemes, showing the interlandmark distances that accounted for the most pronounced shape differences between samples. Because moderate shape changes were large in number, we only report those concerning the ancestors' comparison as a range scatterplot. In both graphs it is pointed out whether the quantitative genetics expectation is verified or not. Moreover, a graphic portrayal summarizing the results displayed and indicating the percentage of verified cases is reported.

RESULTS

Tests performed to evaluate the goodness of the data to perform geometric morphometric analyses confirmed that assumptions like isotropy and extrapolation to the tangent plane are well-accomplished. Concerning isotropy, the percentages of variability explained by the first three principal components computed on the raw data of each group were low (18.61%, 15.37%, and 12.91% for the SA group; 26.16%, 13.16%, and 10.92% for the AA group; 38.79%, 20.11%, and 17.17% for the SMT group; and 21.10%, 18.77%, and 12.68% for the HSJ group), so isotropy of data can be assumed. Moreover, the correlation between Procrustes and Euclidean distances was found to be very strong and highly significant (correlation (uncentered), 1.00; slope, 0.999577; root mean square error, 0.000041). Therefore, statistical analyses were performed on the fitted coordinates.

When geometric morphometrics and multivariate CVA were carried out, results showed a clear differentiation between ancestor groups, while the admixed groups tended to present intermediate values. The canonical variates analysis (Fig. 2 and Table 3) showed that the ancestor groups are separated along the first canonical root, while the colonial ones appear in an intermediate position and show values around the total centroid (Fig. 2a). The ancestor populations display two contrasting morphological patterns. The thin-plate splines (Fig. 2b) reflect differences between the Amerindian ancestor (AA) and the Spanish ancestor (SA) mainly due to an enlargement of the midposterior neurocranium, and to increases in prognathism, facial flattening, and zygoma-

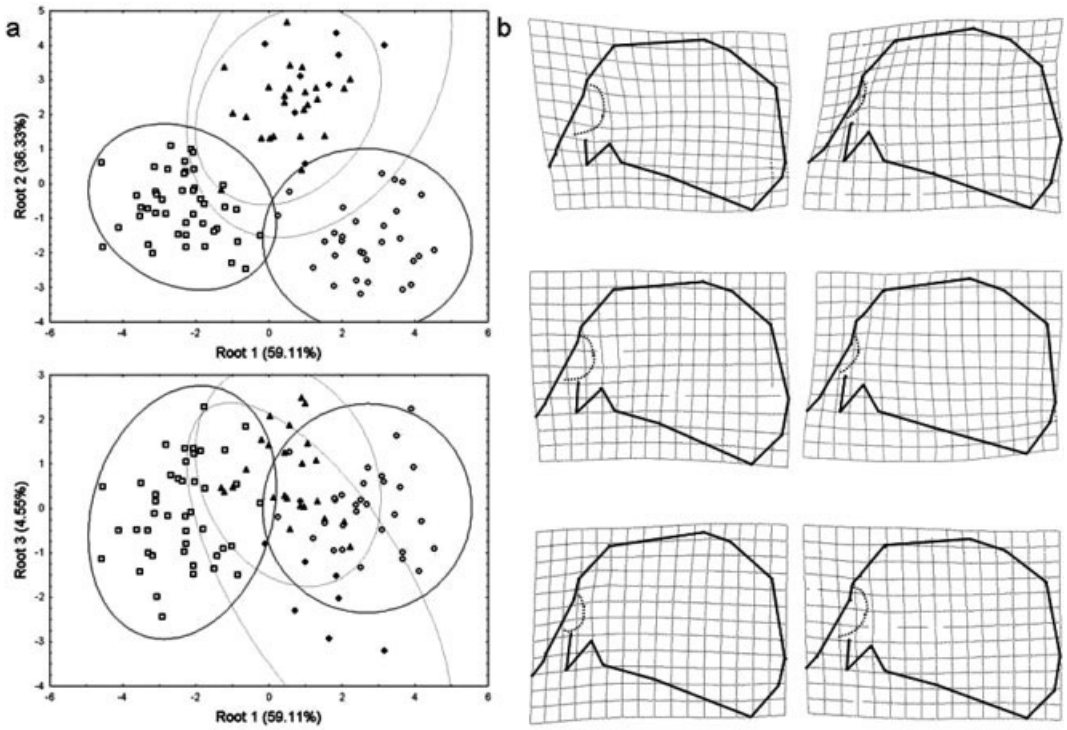

Fig. 2. **a:** Canonical variates analysis. SA, open squares; AA, open circles; SMT, solid diamonds; HSJ, solid triangles. Ellipses account for 95% of within-group variation. Solid lines are used for ancestor series; dashed lines for colonial series. Top, first and second canonical roots; bottom, first and third roots. **b:** Thin-plate splines obtained after regression of partial warps onto first (top), second (center), and third (bottom) canonical roots. Left: Negative canonical values. Right: Positive values.

TABLE 3. Details of CVA analysis of weight matrix[1]

Roots	Eigenvalue	Variance (%)	Canonical R	df	*P*-level
0	4.773853	59.1111	0.909288	102	0.000000
1	2.934131	95.4422	0.863605	66	0.000000
2	0.368090	100.0000	0.518704	32	0.719975

[1] Eigenvalues of successive roots removed, cumulative percentages of variance explained, canonical R values, degrees of freedom, and levels of significance are provided.

TABLE 4. Mahalanobis squared distances and corresponding P-values[1]

	SA	AA	SMT	HSJ
SA	0.00			
AA	27.64	0.00		
SMT	32.10	28.15	0.00	
HSJ	18.36	21.26	7.83, NS	0.00

[1] All distances reported are significant at the 0.0001 level, except 7.83. NS, not significant. Also note that Bonferroni corrected *P*-value was set to 0.008333.

lar development in AA (positive values). The early colonial series, Santa María Texcalac (SMT), shows affinity with Amerindians, while the late colonial group, Hospital San Juan de Dios (HSJ), presents a broader dispersion, overlapping the ranges of variation of the Spanish and Amerindian groups.

The second canonical root, however, does not reflect any intermediate position of the admixed between both parental spectrums of variability, but a separation of them (Fig. 2a). The splines associated with the second canonical root reflect slight changes in the mid- and posterior cranial vault, as well as variation in jugale height (Fig. 2b). When the third root is considered, although the overlap among samples tends to increase, it contributes to segregate the colonial groups. SMT shows slight affinity for the Amerindian craniofacial morphology, and HSJ retains the intermediate position.

Mahalanobis distances (Table 4) showed that ancestor groups were more distant between them than in reference to any of the admixed groups, with the late colonial group being closer to the Spanish ancestor than to the Amerindian one. The closest groups were the two admixed, which were not statically different. However, the distances between the early colonial and any of the two ancestor groups were the greatest. This may be due to the low SMT group size, but it is interesting that besides this, SMT remains closer to its Amerindian ancestor.

In order to explore more accurately the global and localized shape changes responsible for these craniofacial patterns, data were analyzed using EDMA. Major localized shape changes (scaled linear differences $\geq$10% longer or shorter in the reference than in the target shape) are shown in Figure 3. It can be observed that major shape differences are restricted to interlandmark distances measuring cranial vault length, occipital development, and facial flattening. When comparing the two ancestral craniofacial shapes (Fig. 3a), eight interlandmark distances appear to resume major shape changes, which are consistent with the overall shape descriptions reported by the grid deformations and explained above. These distances were further used to test the predictions posed by quantitative genetics theory. Seven major interlandmark distances (87.5%) presented the expected pattern: an intermediate distance for the admixed groups,

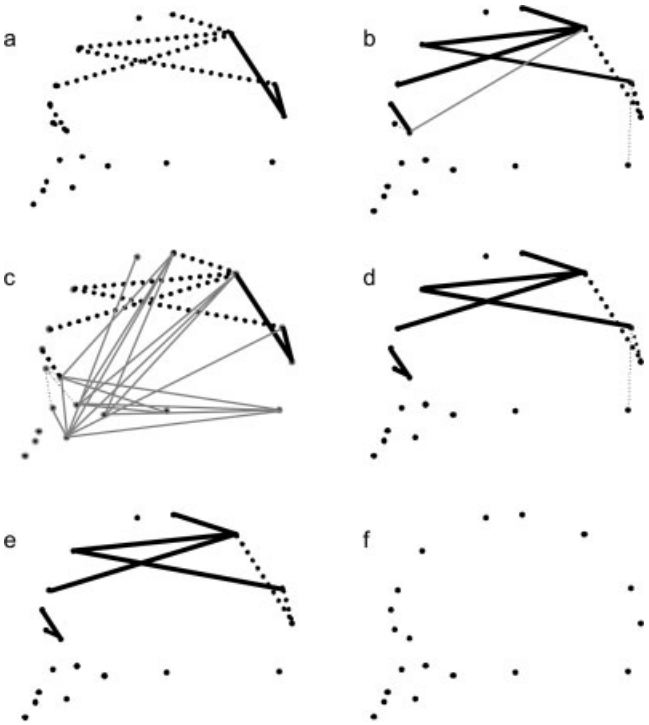

Fig. 3. EDMA mean shape comparisons, highlighting major shape changes. **a:** AA-SA. **b:** SMT-AA. **c:** SMT-SA. **d:** HSJ-AA. **e:** SA-HSJ. **f:** SMT-HSJ. Solid and dashed lines, respectively, indicate scaled linear distances 10% longer or shorter in reference shape (first) than in target shape (second). Black thick lines indicate those distances that fit the expected morphological pattern, whereas grey thin lines indicate those distances that are considered deviations from the quantitative genetics expectations.

compared to that between the two ancestors (Fig. 3b–e). In those distances, when AA was longer than SA, both SMT and HSJ were shorter than AA and longer than SA, and vice versa. Only one distance (nasion–frontomalar orbitale) departed from the expected pattern, being shorter in SMT than in both ancestors. The distances responsible for this pattern include measures of structures that respond to the neural growth pattern, like the cranial vault and the orbit.

Besides these, additional major shape differences were detected in the comparisons between some ancestor and some colonial groups, also reflecting departures from the expected pattern. This is strongly evidenced in the SMT-SA comparison (Fig. 3c), where there are major shape changes which are not detected in the comparison of the two ancestors. For instance, distances from the vertex, inion, and point A to the frontomalar orbitale, zygomaxillar, jugale, and point B play an important role in differentiating even further the mean shapes of SMT and SA, but do not contribute to separate SA and AA. Moreover, note that as a general result, all pairwise shape comparisons are significantly different, except for the pair SMT-HSJ. These results are in accordance with the Mahalanobis distances.

Figure 4 plots moderate shape differences, considering the confidence intervals of those interlandmark distances $\geq$5 or $<$10% longer or shorter in AA than in SA mean shapes. Of 52 interlandmark distances, only 48% fit the expected pattern in both colonials, 21.1% fit in HSJ but not in SMT, 1.9% fit in SMT but not in HSJ, and 28.8% did not fit the pattern expected under the genetic quantitative theory, either in SMT or in HSJ. Thus, the high "predictor efficiency" of major shape changes diminishes from 87.5% to 48% when moderate shape changes are considered. As shown in Figure 4a, more than 50% of the interlandmark distances that fit the expected pattern correspond to neural structures.

Moderate shape changes were broken down into two new categories, to explore more accurately the prediction patterns of the genetic quantitative and cranial integration hypotheses (Fig. 4b). The graph shows that the number of observations that fit the quantitative genetic hypothesis decayed as the magnitude of shape change decreased. Also, note that most moderate changes fall in the category $\geq$5 and $<$8%.

DISCUSSION

When the magnitude of shape difference is high ($\geq$10%), admixed groups tend to present an intermediate position among the ancestral ones, thus fitting the expected coordinated response under quantitative genetics theory. Moreover, all of those distances are mainly located in the neural region. On the other hand, when the magnitude of shape difference is moderate, the number of observations that accomplish the expected pattern diminishes progressively, and the distances involved tend to cover different cranial regions (e.g., combinations of neural-facial structures). Interlandmark distances measuring facial components represent a relatively low percentage of the number of observations that fit the expected pattern. Distances that do not fit the quantitative genetic prediction account for nearly 50% of the total moderate shape changes.

In large-scale human migrations, when migrants settle in an inhabited area, intermating will produce a hybrid population (Wijsman and Cavalli-Sforza, 1984). Although this type of migration may be relatively rare, it can have a marked effect on genetic structure. The contact between Spanish conquerors and Aztec groups inhabiting Mexico in the 16th century can be viewed as the starting point of one of the most well-established large-scale migration processes (Sans, 2000). Hence, the study of admixture through skeletal remains would allow us not only to explore the picture of gene flow among ancestor groups in a given period (represented by the dating of the admixed sample), but also to understand the evolution of the admixture process from a diachronic perspective (Wijsman and Neves, 1986; Stojanowski, 2003, 2004). This evolutionary approach can be achieved when the materials analyzed represent different periods of the admixed group's history.

From a theoretical perspective, cranial phenotypes cannot be considered selectively neutral. As a consequence, and even when some authors demonstrated that a multivariant approach to skull samples can be a good fit to an assumption of selective neutrality (Relethford, 2002; Sparks and Jantz, 2002; González-José et al., 2004), the interpretation of diachronic morphological changes after the contact event must be regarded cautiously. However, several studies based on morphological traits such as skin color pointed out that the analysis of quantitative traits can shed light on the understanding of the combination of microevolutionary agents acting on genetic variability (Sans, 2000).

Quantitative traits may thus reflect more complex processes than the traits determined by single genes. Taking into account the above considerations, morphological

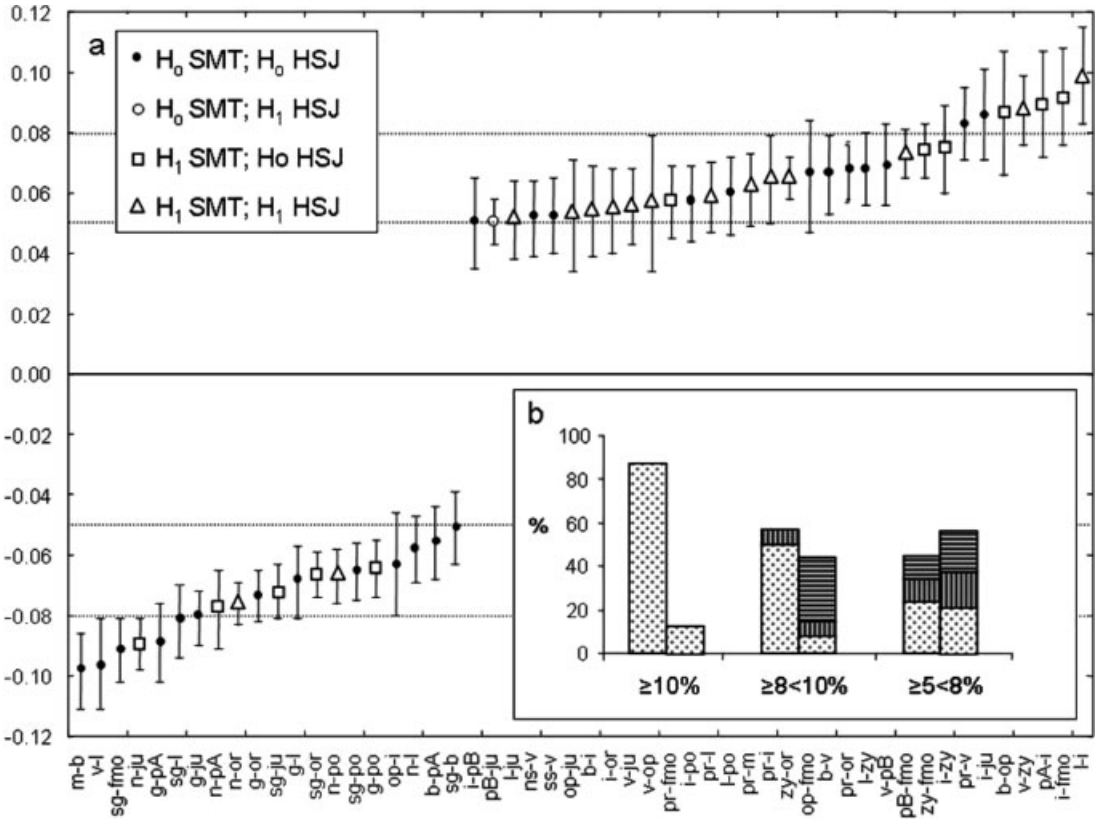

Fig. 4. **a:** Range scatterplot of elements of shape difference matrix, representing moderate shape changes between ancestor series. H_0, expected pattern observed; H_1, expected pattern not observed. **b:** Percentages of fitted observations in major and moderate shape changes. Left column shows percentages of distances that fit quantitative genetics prediction, whereas right column shows percentages that did not. Each column is subdivided in percentages of distances measuring structures derived from neural growth pattern (dashed region), from somatic growth pattern (vertical lines), and comprising both structures (horizontal lines). See Table 2 for landmark abbreviations.

traits may be considered useful tools to explore patterns of population structure and gene flow. For instance, Relethford (2004), in a revision of the Boas debate over the relative effects of cranial plasticity resulting from migration to a new environment, found a fit of global craniometric variation to the isolation-by-distance model, assuming a neutral model of quantitative variation. These results show that despite environmental influences (both developmental plasticity and climatic adaptation) on craniometric variation, it still reflects the underlying patterns of population structure and history (Relethford, 2004). Thus, the overall phenotypic information obtained from craniometric data analyses can be used to study gene flow, as well as developmental plasticity and long-term adaptation, depending on the specific scope of the study and the analytical approach (Jantz, 1974; Key and Jantz, 1981).

Here we present a geometric morphometric comparative study of two ancestral and two admixed groups from Mexico's Central Valley, in order to perform a quantitative approach to the morphological outcome of the gene flow resulting from the Spanish-Amerindian contact. Furthermore, we intend to evaluate if admixture levels inferred after morphometric distances were in accordance with the final step of the gene flow process, i.e., the rates of molecular admixture estimated in the modern population. Finally, we looked for potential, localized cranial structures responsible for departures from the expected morphological pattern of the admixed groups.

According to our data, the main differences among Amerindians from Mexico's Central Valley and Spaniards can be described by an enlargement of the midposterior neurocranium, increased prognathism, facial flattening, and zygomalar development in the former with respect to the latter. On the one hand, skulls from the early colonial series tend to show morphological traits characterizing Amerindians but not completely overlapping their range of variation, and slightly differing toward the Spanish morphology. On the other hand, the late colonial group shows a more intermediate (and internally diverse) morphology. Note that these results depend on the samples as well as on the periods considered in this work, so that they cannot be applied or generalized to the whole Mexican territory. Actually, the impact of admixture on Amerindian Mexican populations differed among regions.

In summary, our results suggest that at least with the samples and periods considered in this paper, colonial samples occupy an intermediate position between both ancestral populations, with the early colonial lying nearer to the Amerindian centroid, and the late colonial closer to the Spanish centroid (Fig. 2). Given the progressive demographic impact of Spanish individuals on the composition of the Mexican population (Crawford, 1998; Salzano and Bortolini, 2002), this is expected. In fact, under a model of increased large-scale presence of the Spanish ancestors over the inhabited area, the early

admixed group is expected to show more affinity to the Amerindian centroid, while the later is expected to approach the Spanish centroid.

The admixture levels inferred in this region based on morphometric distances are in accordance with the final step of the gene flow process. The genetic composition of the modern population of Mexico inferred after molecular markers (Cerda-Flores et al., 2002) shows evidence of admixture with predominantly and nearly equivalent Spanish (50%) and Amerindian (49%) contributions, with an African contribution at around 1%. The position of the late colonial group (HSJ) in relation to both ancestors reflects that, by the end of the 19th century, the colonial population of Mexico was almost equally shaped by the Spanish and Amerindian groups. HSJ represents the immediate anterior step to the modern genetic composition observed by Cerda-Flores et al. (2002) in Mexico City.

Yet despite this general congruence, admixed groups do not show an exact intermediate position in terms of craniofacial shape (Fig. 2). For instance, the second canonical root reflects that the colonial groups present a "third phenotype." Moreover, the EDMA analysis shows that a number of major and moderate shape changes can be considered deviations from quantitative genetics theory. Those departures suggest a phenotypic pattern inherent to admixed populations, the cause of which might be found in a microevolutionary force other than gene flow.

Morphological integration is a key concept in evolutionary morphology that must be considered if a deeper comprehension of skull biology is to be achieved. Due to its variety of functional requirements and growths patterns, the skull can be viewed as a complex morphological structure (Pucciarelli et al., 1990). Thus, to analyze and further understand its biology, as well as the developmental mechanisms and microevolutionary processes by which its phenotypic variation is expressed, morphological integration between structural components related by either developmental or functional criteria must be considered (Olson and Miller, 1958; Marroig and Cheverud, 2001; Bookstein et al., 2003). The skull is considered to be made up of several interdependent structures which behave as an integrated unit. Such structures were identified following a general pattern of mammalian craniofacial growth and development (Cheverud, 1995): the neural structures (i.e., the cranial vault, cranial base, and orbit) and somatic structures (such as the face). Lieberman et al. (2000a,b) suggested that the cranial base strongly influences components of the primate skull during growth and development; unfortunately, our data are limited to specimens' lateral views, and the cranial base is not completely represented in our set of landmarks.

When testing for deviations of quantitative genetics with our data and taking morphological integration into account, the results show that neural structures tend to behave as an integrated unit, showing a coordinated response to shape changes. Since the null hypothesis of intermediate craniofacial shape for admixed groups is found at such regions, their phenotypic expression should be considered as being mainly influenced by the gene flow and admixture events experienced among both ancestors. Conversely, the splines correlated with the second canonical root (Fig. 2), as well as the interlandmark distances (Fig. 4), reflect that localized structures departing from the expected intermediate shape are mainly derived from somatic growth patterns, such as the lower and upper face. These regions could be responsible for a particular differentiated morphology characterizing colonial groups, and could result from a different microevolutionary force. Finally, it is interesting that the distances that could not be assigned to a particular developmental pattern appeared to introduce "noise" and blur the effects of cranial integration.

Future research should focus on detecting at which ontogenetic stage the traits characterizing admixed groups appear. If those morphological features were established early in ontogeny, then they would reflect an adaptive origin and consequent fixation. Conversely, if they tend to appear during the adult stage, they should be interpreted as the final result of plastic responses to environmental stressors. Other microevolutionary agents like natural selection, both internal and external (Lande, 1979), as well as plastic responses to mechanical loadings, can generate shape changes in some structures. Furthermore, different structures are likely to display different degrees of plasticity, with some regions being more sensitive than others to different environmental agents (Lieberman et al., 2000b).

CONCLUSIONS

The analyses presented here reveal that when considered from the perspective of craniofacial shape, admixture processes are well-described in general terms. The global morphology of the admixed groups falls between the range of variation of the ancestral groups. The results are in accordance with historic, demographic, and genetic evidence. Thus, when global shape is considered, the null hypothesis could be accepted. However, when localized structures (mainly facial) are taken into account, deviations from the expected pattern are detected, and the null hypothesis should be rejected. Therefore, the data show complicated and integrated shape changes which cannot be exclusively explained by a gene flow model.

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LITERATURE CITED

Aguirre Beltrán G. 1972. La población negra de México. Estudio etnohistórico. Mexico City: Fondo de Cultura Económica.

Baume R, Crawford MH. 1978. Discrete dental traits in four Tlaxcaltecan Mexican populations. Am J Phys Anthropol 49: 351–360.

Bookstein FL. 1991. Morphometric tools for landmark data. Cambridge: Cambridge University Press.

Bookstein FL. 1996. Combining the tools of geometric morphometrics. In: Marcus LF, Corti M, Loy A, Naylor GJP, Slice DE, editors. Advances in morphometrics. New York: Plenum Press. p 131–151.

Bookstein FL, Gunz P, Mitteroecker P, Prossinger H, Schaefer K, Seidler H. 2003. Cranial integration in *Homo*: singular warps analysis of the midsagittal plane in ontogeny and evolution. J Hum Evol 44:167–187.

Bräuer G. 1988. Osteometrie. In: Martin R, Knussmann R, editors. Handbuch der Vergleichenden. Biologie des Menchen. Stuttgart: Gustav Fischer Verlag. p 160–232.

Cerda-Flores RM, Villalobos-Torres MC, Barrera-Saldana HA, Cortes-Prieto LM, Barajas LO, Rivas F, Carracedo A, Zhong Y, Barton SA, Chakraborty R. 2002. Genetic admixture in three Mexican mestizo populations based on DS180 and HLA-DQA1 loci. Am J Hum Biol 14:257–263.

Chakraborty R. 1986. Gene admixture in human populations: models and predictions. Yrbk Phys Anthropol 29:1–43.

Chakraborty R. 1990. Quantitative traits in relation to population structure: why and how are they used and what do they imply? Hum Biol 62:147–162.

Cheverud JM. 1995. Morphological integration in the saddle-back tamarin (*Saguinus fuscicollis*) cranium. Am Nat 145:63–89.

Cole TM III. 2002. WinEDMA: software for Euclidean distance matrix analysis. Version 1.0.1 beta. Kansas City: Kansas City School of Medicine, University of Missouri.

Crawford MH. 1998. The origins of Native Americans. Evidence from anthropological genetics. Cambridge: Cambridge University Press.

Crawford MH, Devor EJ. 1980. Population structure and admixture in transplanted Tlaxcaltecan populations. Am J Phys Anthropol 52:485–490.

Díez de la Calle J. 1932. Memorial y noticias sacras y reales de las Indias Occidentales. Mexico City: Bibliófilos Mexicanos.

Domínguez Olivier Y. 1984. Los dermatoglifos como indicadores de mestizaje en México. Tesis de Licenciatura. Mexico City: Escuela Nacional de Antropología e Historia.

Dryden IL, Mardia KV. 1998. Statistical shape analysis. Chichester: John Wiley and Sons.

Faulhaber J. 1955. Antropología física de Veracruz. Mexico City: Gobierno de Veracruz.

Futuyma DJ. 1986. Evolutionary biology. Sunderland, MA: Sinauer Associates.

Garralda MD, Mesa MS. 1984. Variabilité morphologique dans la Péninsule Ibérique: épipaleolithique-âge ancien. Bull Mem Soc Anthropol Paris 1:207–220.

García Martínez B. 1990. Pueblos de Indios, pueblos de Castas: new settlements and traditional corporate organization in eighteenth century New Spain in the Indian community of colonial Mexico. Amsterdam: CEDLA.

Gibson C. 1961. Los Aztecas bajo el dominio español (1513–1810). Mexico City: Siglo Veintiuno.

Giesen EB, Ding M, Dalstra M, van Eijden TM. 2003. Reduced mechanical load decreases the density, stiffness, and strength of cancellous bone of the mandibular condyle. Clin Biomech 18:358–363.

González-José R, Van der Molen S, González-Pérez 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.

Goodall CR. 1991. Procrustes methods in the statistical analysis of shape. J R Stat Soc [B] 53:285–339.

Jantz RL. 1974. Microevolutionary change in Arikara crania: a multivariate analysis. Am J Phys Anthropol 38:15–26.

Key P, Jantz RL. 1981. A multivariate analysis of temporal change in Arikara craniometrics: a methodological approach. Am J Phys Anthropol 55:247–259.

Kiliaridis S. 1995. Masticatory muscle influence on craniofacial growth. Acta Odontol Scand 53:196–202.

Konigsberg LW. 2000. Quantitative variation and genetics. In: Stinson S, Bogin B, Huss-Ashmore R, O'Rourke D, editors. Human biology: an evolutionary and biocultural perspective. New York: Wiley-Liss. p 135–162.

Lalueza Fox C, González Martín A, Vives Civit S. 1996. Cranial variation in the Iberian Peninsula and the Balearic islands: inferences about the history of the population. Am J Phys Anthropol 99:413–428.

Lande R. 1979. Quantitative genetic analysis of multivariate evolution, applied to brain: body size allometry. Evolution 33:402–416.

Lele SR, Cole TM III. 1996. A new test for shape differences when variance-covariance matrices are unequal. J Hum Evol 31:193–212.

Lele SR, Richtsmeier JT. 1995. Euclidean distance matrix analysis: confidence intervals for form and growth differences. Am J Phys Anthropol 98:73–86.

Lele SR, Richtsmeier JT. 2001. An invariant approach to statistical analysis of shapes. Boca Raton: Chapman and Hall.

Lieberman DE. 1997. Making behavioral and phylogenetic inferences from hominid fossils: considering the developmental influence of mechanical forces. Annu Rev Anthropol 26:185–210.

Lieberman DE, Ross CR, Ravosa M. 2000a. The primate cranial base: ontogeny, function and integration. Yrbk Phys Anthropol 43:117–169.

Lieberman DE, Mowbray KM, Pearson OMJ. 2000b. Basicranial influences on overall cranial shape. J Hum Evol 38:291–315.

Lieberman DE, Krovitz GE, Yates FW, Devlin M, St. Claire M. 2004. Effects of food processing on masticatory strain and craniofacial growth in a retrognathic face. J Hum Evol 46:655–677.

Lisker R, Pérez RB, Granados J, Babinsky V, Rubens J, Armendares S, Buentello L. 1986. Gene frequencies and admixture estimates in a Mexico City population. Am J Phys Anthropol 71:203–207.

Lisker R, Ramírez E, Babinsky V. 1996. Genetic structure of autochthonous populations of Meso-America: Mexico. Hum Biol 6:395–404.

Liu J, Mercer JM, Stam LF, Gibson GC, Zeng ZB, Laurie CC. 1996. Genetic analysis of a morphological shape difference in the male genitalia of *Drosophila simulans* and *D. mauritania*. Genetics 142:1129–1145.

López Alonso S. 1990. Datos dermatoglíficos de una muestra de población mestiza de la Ciudad de Móxico. Est Anthropol Biol 5:275–286.

Manly BFJ. 1994. Multivariate statistical methods. A primer. London: Chapman and Hall.

Marcus LF, Corti M, Loy A, Naylor GJP, Slice DE. 1996. Advances in morphometrics. New York: Plenum Press.

Márquez Morfín L. 1993. La evolución cuantitativa de la población novohispana: siglos XVI, XVII y XVIII. In: Arenzana A, editor. El poblamiento de México. Una visión histórico-demográfica. El México colonial. Mexico City: Secretaría de Gobernación, Consejo Nacional de Población. p 36–63.

Marroig G, Cheverud JM. 2001. A comparison of phenotypic variation and covariation patterns and the role of phylogeny, ecology, and ontogeny during cranial evolution of New World monkeys. Evolution 55:2576–2600.

Olson EC, Miller RL. 1958. Morphological Integration. Chicago: University of Chicago Press.

Pucciarelli H, Dressino V, Niveiro MH. 1990. Changes in skull components of the squirrel monkey evoked by growth and nutrition: an experimental study. Am J Phys Anthropol 81: 535–543.

Pucciarelli H, Muñe MC, Oyhenart EE, Orden AB, Villanueva ME, Rodríguez RR, Pons ER. 2000. Growth of skeletal components in the young squirrel monkey (*Saimiri sciureus boliviensis*): a longitudinal experiment. Am J Phys Anthropol 112:57–68.

Relethford JH. 2002. Apportionment of global human genetic diversity based on craniometrics and skin color. Am J Phys Anthropol 118:393–398.

Relethford JH. 2004. Boas and beyond: migration and craniometric variation. Am J Hum Biol 16:379–386.

Richtsmeier JT, Burke Deleon V, Lele SR. 2002. The promise of geometric morhometrics. Yrbk Phys Anthropol 45:63–91.

Rohlf FJ. 1990. Rotational fit (Procrustes) methods. In: Rohlf FJ, Bookstein FL, editors. Proceedings of the Michigan Morphometrics Workshop. Ann Arbor: University of Michigan Museum of Zoology. p 227–236.

Rohlf FJ. 1998a. tpsDig. New York: Department of Ecology and Evolution, State University of New York at Stony Brook.

Rohlf FJ. 1998b. tpsSmall version 1.20. New York: Department of Ecology and Evolution, State University of New York at Stony Brook.

Rohlf FJ. 1998c. On applications of geometric morphometrics to studies of ontogeny and phylogeny. Syst Biol 47:147–158.

Rohlf FJ. 2003a. tpsRelw version 1.35. New York: Department of Ecology and Evolution, State University of New York at Stony Brook.

Rohlf FJ. 2003b. tpsRegr version 1.28. New York: Department of Ecology and Evolution, State University of New York at Stony Brook.

Rohlf FJ, Marcus LF. 1993. A revolution in morphometrics. Tree 8:129–132.

Rohlf FJ, Slice DE. 1990. Extensions of Procrustes method for the optimal superimposition of landmarks. Syst Zool 39:40–59.

Ross AH, Slice DE, Ubelaker DH, Falsetti AB. 2004. Population affinities of 19th century Cuban crania: implications for identification criteria in South Florida Cuban Americans. J Forensic Sci 49:1–6.

Salzano FM, Bortolini MC. 2002. The evolution and genetics of Latin American populations. Cambridge: Cambridge University Press.

Sánchez Albornoz N. 1973. La población de América Latina. Desde los tiempos precolombinos al año 2000. Madrid: Alianza Universidad.

Sans M. 2000. Admixture studies in Latin America: from the 20th to the 21st century. Hum Biol 72:155–177.

Serrano C. 1982. Dermatoglifos de coras, huicholes y mestizos de la Sierra Norte de Nayarit, México. Est Antropol Biol 1:162.

Sparks CS, Jantz RL. 2002. A reassessment of human cranial plasticity: Boas revisited. Proc Natl Acad Sci USA 99:14636–14639.

Stojanowski CM. 2003. Differential phenotypic variability among the Apalachee mission populations of La Florida: a diachronic perspective. Am J Phys Anthropol 120:352–363.

Stojanowski CM. 2004. Population history of native groups in pre- and postcontact Spanish Florida: aggregation, gene flow, and genetic drift on the Southeastern US Atlantic coast. Am J Phys Anthropol 123:316–332.

Tiburcio V, Romero A, De Garay AL. 1978. Gene frequencies and racial admixture in a Mestizo population from Mexico City. Ann Hum Biol 5:131–138.

Velasco MP. 1993. La migración ibérica y africana: características e impactos regionales. In: Arenzana A, editor. El poblamiento de México. Una visión histórico-demográfica. El México colonial. Mexico City: Secretaría de Gobernación, Consejo Nacional de Población. p 64–85.

Wijsman EM, Cavalli-Sforza LL. 1984. Migration and genetic population structure with special reference to humans. Annu Rev Ecol Syst 15:279–301.

Wijsman EM, Neves WA. 1986. The use of nonmetric variation in estimating human population admixture: a test case with Brazilian blacks, whites, and mulattos. Am J Phys Anthropol 70:395–405.

Wood B, Lieberman DE. 2001. Craniodental variation in *Paranthropus boisei*: a developmental and functional perspective. Am J Phys Anthropol 116:13–25.