

Ontogenetic Allometry and Cranial Shape Diversification Among Human Populations From South America

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ABSTRACT

Modifications of ontogenetic allometries play an important role in patterning the shape differentiation among populations. This study evaluates the influence of size variation on craniofacial shape disparity among human populations from South America and assesses whether the morphological disparity observed at the interpopulation level resulted from a variable extension of the same ontogenetic allometry, or whether it arose as a result of divergences in the pattern of size-related shape changes. The size and shape of 282 adult and subadult crania were described by geometric morphometric-based techniques. Multivariate regressions were used to evaluate the influence of size on shape differentiation between and within populations, and phylogenetic comparative methods were used to take into account the shared evolutionary history among populations. The phylogenetic generalized least-squares models showed that size accounts for a significant amount of shape variation among populations for the vault and face but not for the base, suggesting that the three modules did not exhibit a uniform response to changes in overall growth. The common slope test indicated that patterns of evolutionary and ontogenetic allometry for the vault and face were similar and characterized by a heightening of the face and a lengthening of the vault with increasing size. The conservation of the same pattern of shape changes with size suggests that differences in the extent of growth contributed to the interpopulation cranial shape variation and that certain directions of morphological change were favored by the trait covariation along ontogeny. *Anat Rec*, 00:000–000, 2011. © 2011 Wiley-Liss, Inc.

Key words: ontogenetic and evolutionary allometry; cranial modules; geometric morphometrics; growth

INTRODUCTION

Variation in morphological traits originates either from the direct effects of ecological and evolutionary factors on specific traits or from correlated responses to factors that affect other traits (Lande, 1979; Cheverud, 1982). Such correlated changes arise as a consequence of developmental and functional interactions among traits and pleiotropic effects during organism ontogeny (Frankino et al., 2005; Hendrikse et al., 2007; Shingleton et al., 2007; Jamniczky et al., 2010). Particularly, variation in the absolute size of the total organism or specific parts can generate proportional changes in the dimensions of particular anatomical traits as well as correlated physiological and behavioral changes, a phenomena referred to as allometry (Gould, 1966).

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In the last few years, there has been a renewed interest in evaluating the association of shape changes—that is, changes in the relative spatial position of anatomical traits—in single or multiple traits with the variation in overall size across ontogenetic and evolutionary scales (Klingenberg, 2010). A complex picture emerges from these studies, since in some cases the shape variation associated with the evolution of size seems to be channeled in particular directions, resulting from extensions or truncations along common ontogenetic allometries (Marroig and Cheverud, 2001; Cardini and Thorington, 2006; Marroig, 2007). In contrast, other studies found that the pattern of size-related shape changes during ontogeny is not always conserved, and thus, the direction of the ontogenetic allometries can be altered resulting in morphological differences among populations, and in turn, among species (Strand Vidarsdottir et al., 2002; Cobb and O'Higgins, 2004; Gerber et al., 2004; Mitteroecker et al., 2004; Adams and Nistri, 2010; Willson and Sanchez Villagra, 2010). Despite their discrepancies, these studies remark the importance of growth changes during ontogeny in patterning the shape differentiation among populations and species, which may be related to ecological or evolutionary factors (Klingenberg, 2010).

Although the importance of modifications of the relative growth trajectories in originating population differentiation is recognized, the processes involved in the origin of morphological variation among modern humans have been mainly discussed by analyzing adult individuals, and thus the ontogenetic basis of such differentiation remains largely untested. In this study, we focused on the influence of size on cranial shape disparity among human populations from southern South America and assesses whether a differential length of ontogenetic trajectories among populations (i.e., differential growth) might have been responsible for the shape variation observed in this region. The use of American human crania is advantageous as these populations have a recent and single origin about 12,500–13,000 years ago—they share the same haplogroups for mtDNA and Y chromosome as well as alleles at specific microsatellite loci that are not found in any Old World population— and the genetic diversity is reduced (Goebel et al., 2008; Steele and Politis, 2009). Additionally, the molecular variation among these populations is relatively well known, showing a latitudinal gradient with the mitochondrial haplogroups A and B decreasing from North to South and the haplogroups C and D increasing in the same direction (Moraga et al., 2000). The knowledge about the evolutionary relationships among these populations allows for a greater control over other factors, such as the environmental variables, that might have influenced cranial size and shape. Previous studies have found large differences in adult body and cranial size among South American populations, which seem to be highly correlated with ecological variables, and particularly the diet (Béguelin, 2009; Perez and Monteiro, 2009; Bernal et al., 2006, 2010). The broad pattern found suggests that adults belonging to agriculturalists possessed the smallest crania, with shorter and less robust faces, compared with the hunter-gatherers from the same region. Moreover, the influence of environmental factors is supported by the fact that the level of diversification involved larger morphological changes than expected based on genetic drift alone, given the short period of evolutionary

time in which they occurred (Perez and Monteiro, 2009). These particular characteristics make the region a suitable setting in which to analyse the contribution of allometry to shape differentiation among populations and to discuss the factors that drove such changes.

To evaluate the role of size in cranial shape differentiation, we first studied the pattern of evolutionary allometry in the cranium by analyzing the relationship between size and shape variation among populations. In this sense, evolutionary allometry refers to morphological differences associated with divergence in size at different taxonomic levels, intra and interspecific, and regardless of whether the taxa under study are linked by ancestor-descendant or sister group relationships (Klingenberg, 1998; Gustafsson and Lindenfors, 2004). Because populations might be similar in shape due to shared evolutionary history, we used comparative methods that incorporate the evolutionary relatedness structure to test the association between shape and size variables (Felsenstein, 1985). Then, we compared the patterns of size-related shape changes among and within populations to evaluate whether the morphological variation observed among populations resulted from a variable extension of the same ontogenetic allometry, or whether they arose as a result of divergences in the pattern of size-related shape changes during ontogeny. Because the cranium consists of recognisable parts or modules that are coherent according to their developmental origins, structure, and function, we performed these analyses separating the cranium into the three main modules in which it is usually divided (base, face, and vault; Sperber, 2001). This is relevant for this study because the relative independence of the modules can result in diverse patterns of covariation with size within the skull (Mitteroecker et al., 2005).

MATERIALS AND METHODS

Samples

We studied 12 samples of adults belonging to prehistoric populations from southern South America (ca. 200–1,500 years BP) that came from different geographic and ecological regions spread over 3,500 km (Table 1; Fig. 1). The northernmost samples are composed of two samples of agriculturalists (i.e., populations that based their diets mainly on domesticated plants; PG and SJ) and two terrestrial hunter-gatherers (Cha and Del), the central samples are represented by two horticulturalists (i.e., populations that incorporated a lower proportion of domesticated plants along with wild plants and animals, Smith, 2001; Ar and Pa) and two terrestrial hunter-gatherers (ChV and SCCh), whereas the south samples are composed by two terrestrial (SP and TF) and two maritime hunter-gatherers (AI and BC) (see references in Perez et al., 2011).

We also analysed ontogenetic series of four of these populations (ChV, Pa, PG, SP-TF) to compare the patterns of evolutionary and ontogenetic allometries among them. The ontogenetic series include individuals of both sexes from 4 years of age to adults. Individuals were assigned as adults if their crania showed the basioccipital synchondrosis obliterated, while those crania showing this suture opened were assigned as subadults. The age of the subadults was estimated on the basis of the standard of formation and eruption pattern of upper and

TABLE 1. Sample composition of adult and subadult individuals

Samples ^a	Abbr.	Region	Adults			Subadults	Years BP ^b
			<i>n</i>	Male (%)	Female (%)		
Pampa Grande	PG	Northwest-Chaco	15	46	54	9	500–1500
Chaco	Cha	Chaco	18	56	44		200–1000
San Juan-North Mendoza	SJ	Cuyo	15	54	46		400–1500
Delta	Del	Pampean Region	17	53	47		400–1000
Pampa	Pa	Pampean Region	20	45	55	21	200–1000
Araucania	Ar	Araucanian Region	17	53	47		200–1000
Chubut valley	ChV	Continental Patagonia	23	44	56	15	400–1500
Santa Cruz-Chubut	SCCh	Continental Patagonia	21	43	57		300–1500
South Patagonia	SP	Continental Patagonia	18	61	39	9	200–1500
Tierra del Fuego	TF	Insular Patagonia	21	57	43		200–1500
Austral Island	AI	Insular Patagonia	21	57	43		200–1500
Beagle channel	BC	Insular Patagonia	20	45	55		200–1500
Total			228	–	–		

^aThe specimens are housed at Museo de La Plata (La Plata, Argentina), Museo Etnográfico “J. B. Ambrosetti” (Buenos Aires, Argentina), Museo Regional Provincial ‘Padre Manuel Jesús Molina’ (Río Gallegos, Argentina), Museo del Fin del Mundo (Ushuaia, Argentina), Instituto Nacional de Antropología y Pensamiento Latinoamericano (Buenos Aires, Argentina), Museo de Historia Natural (Santiago, Chile) and Instituto de la Patagonia Austral (Punta Arenas, Chile).

^bApproximate sample ages according to radiocarbon dating and contextual information.

lower teeth proposed by Ubelaker (1989) for Amerindian populations. The four ontogenetic series comprise individuals aged from 4 to 15 years (Table 2). The age distribution of subadults between samples was compared using a χ^2 test with Bonferroni correction for multiple comparisons ($P = 0.05/6 = 0.0084$). Only the comparison between Pa and SP-TF samples was significantly different ($\chi^2 = 24.14$; $P = 0.0083$).

Males and females were pooled for the comparisons reported herein. To avoid a possible bias due to sexual dimorphism in size, a similar percentage of adult males and females were included in each sample (Table 1). Sexual dimorphism in craniofacial traits in subadults is non significant, so it is not expected that this factor biases the analysis of ontogenetic allometries.

Morphometric Analyses

Cranial traits were captured from digital images as 2D coordinates for landmarks and semilandmarks in frontal, lateral and base views, following the procedure previously described in Perez and Monteiro (2009) (see Fig. 2 and Supporting Information S1). Images of the crania were obtained with an Olympus SP 350 digital camera. For frontal view images, the crania were positioned in the Frankfurt plane, and the camera lens was located in the coronal plane; the images were taken at 250 mm from the prosthion point. For base view images, the photographs were taken at 250 mm from the occlusal surface, placing the cranium in the perpendicular plane and the camera lens in the midsagittal plane. For lateral view images, the crania were positioned in the Frankfurt plane, and the camera lens was placed parallel to the sagittal plane. The images from lateral views were taken at a 300 mm distance from the euryon point. The coordinates used defined the point configurations of the face (Fig. 2a), vault (Fig. 2b) and cranial base (Fig. 2c), which were analysed separately. A scale was placed in every image to measure the actual size of the structures analysed. Previous studies have concluded that, given a careful choice of landmarks, two-dimensional

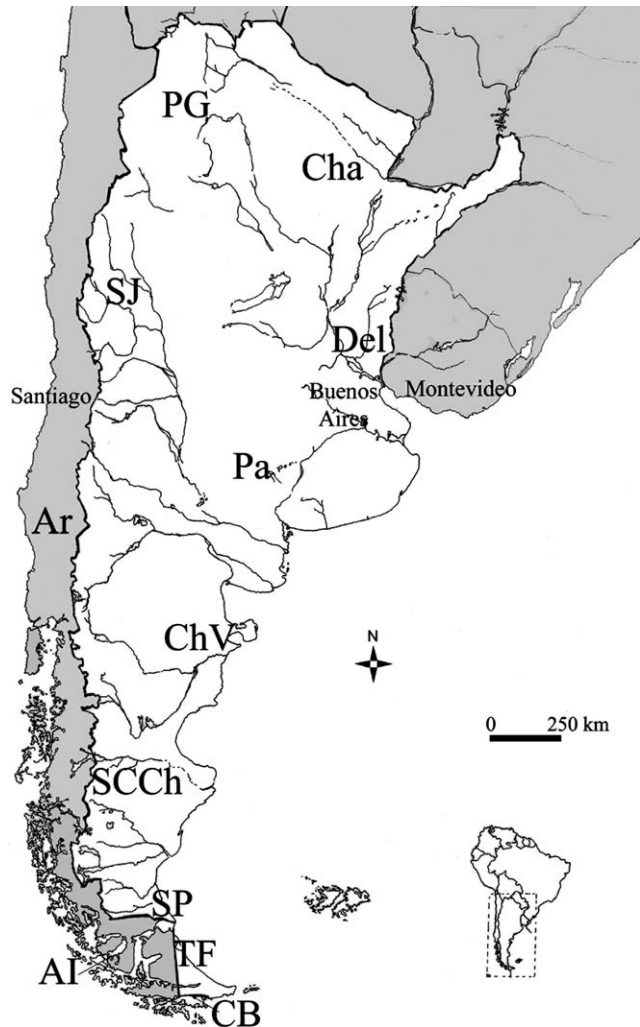


Fig. 1. Map depicting the central geographic location of the samples analyzed. Abbreviations are shown in Table 1.

TABLE 2. Age distribution of the subadult samples

Sample	4–4.9 yrs	5–9.9 yrs	10–14.9 yrs
PG	2	5	2
Pa	2	22	8
ChV	2	6	7
SP-TF	1	3	5
Total	7	25	22

landmarks are fairly accurate descriptors of the size and shape variation of inherently three-dimensional structures, such as the mammalian cranium (Cardini and Thorington, 2006; Percival et al., 2009). The coordinates of landmarks and semilandmarks were recorded using tpsDIG 2.10 software (Rohlf, 2009).

To estimate the precision of the data collected, the intra- and inter-observer error associated with the placement of point coordinates and with cranium orientation in the Frankfurt horizontal plane were previously evaluated (Perez and Monteiro, 2009).

Landmarks and semilandmarks were aligned using a generalized Procrustes analysis (Rohlf and Slice, 1990). Additionally, semilandmarks were slid because the curves or contours that they describe should be homologous from subject to subject, whereas their individual points need not be (Bookstein et al., 2002). We employed the minimum Procrustes distance criterion, which removes the difference along the curve in semilandmark positions between the reference form and each specimen by estimating the direction tangential to the curve and removing the component of the difference that lies along this tangent (Perez et al., 2006). The semilandmarks along a curve are aligned so that the semilandmarks of each specimen lie along the lines perpendicular to the curve that passes through the corresponding semilandmarks on the reference form. In this study, tpsRelw 1.44 (Rohlf, 2009) was used to align the semilandmarks. The coordinates aligned by this procedure (Procrustes shape coordinates) were used to evaluate shape differences between specimens. Additionally, shape differences between specimens and samples were studied using principal components analysis (PCA) obtained from the aligned coordinates (Bookstein, 1991).

The centroid size, defined as the square root of the summed squared distances from all landmarks and semilandmarks to the configuration centroid, was used as a size measurement (Bookstein, 1991). We estimated an overall size measure for the cranium as the sum of the centroid sizes from the face, vault, and base point configurations. We used this measure, instead of the centroid sizes for each of the three modules, because we were interested in evaluating whether the shape changes of these modules were associated with the overall increase in size. In this sense, the composite measurement used is a better estimation of overall cranial size.

Statistical Analyses

Allometries are traditionally analysed using plots of the size of particular traits against the overall body size, whose relationship is usually modelled through a linear equation of the log transformed variables if there are only two variables or through a PCA of the variance-co-

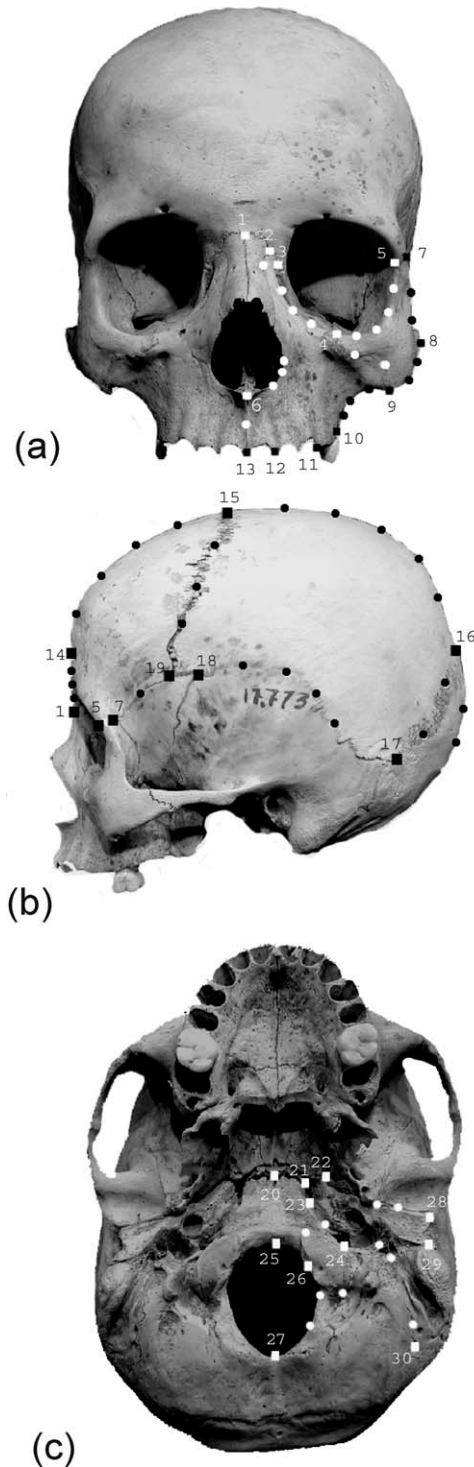


Fig. 2. Allocated reference points are displayed with different symbols. Landmarks are represented as squares, whereas semilandmarks are represented as circles on the face (a), vault (b), and base (c). A list of anatomical landmarks used (numbers 1–30) is provided as Supporting Information (S1).

variance matrix of log-transformed distances for multivariate allometry (Klingenberg, 1996). The approach used within geometric morphometrics differs from the

traditional one because the shape and size are analyzed separately, and thus the allometry is usually studied by multivariate regressions of Procrustes shape coordinates or relative warps on centroid size (Klingenberg, 1996; Mitteroecker et al., 2005).

Analysis of evolutionary allometry. To evaluate the influence of size on shape differentiation between populations, we first explored the relationship between the consensus or mean shape coordinates and the mean centroid size of adult individuals using an ordinary least square (OLS) model. OLS assumes that the error term is the identity matrix (Rohlf, 2001). The proportion of size-related shape change (called “degree of allometry”) was measured as one minus the percent unexplained by the regression model, computed using the Procrustes distance.

Because the use of outline methods poses difficulties in regression analysis due to the large number of semilandmarks per individual needed to describe outlines and to the representation of semilandmark points by two coordinates, the first principal components accounting for 80% of the explained variation were also used as shape variables (Sheets et al., 2006). The significance of the regression models was assessed using the *F*-statistic. Visualizations of the pattern of shape changes for evolutionary allometry were performed through deformation grids obtained from the regression of Procrustes shape coordinates on cranial size.

In addition, we fitted the consensus shape variation to the cranial size using a phylogenetic generalized least-squares model (PGLS) to account for evolutionary non-independence among populations (Rohlf, 2001). PGLS assumes that the error term has a covariance matrix (*C*) derived from the evolutionary relationships among groups. We used a covariance matrix based on a neighbour-joining tree assuming a Brownian model (Rohlf, 2001). The neighbour-joining tree was estimated using Euclidean mtDNA distances between groups (see Supporting Information S2).

Analysis of ontogenetic allometry. We estimated the ontogenetic allometric trajectories of each sample independently and the pooled within-group allometry using a multivariate regression (OLS) of shape variables onto centroid size (Zelditch et al., 2000; Cardini and Thorington, 2006; Drake and Klingenberg, 2008). Both the full set of Procrustes shape coordinates and the first principal components accounting for 80% of explained variation were used as shape variables. The pattern of shape variation for ontogenetic allometry was also depicted by using deformation grids obtained from the regression of Procrustes shape coordinates on cranial size.

Differences in independent ontogenetic allometric trajectories among samples were tested using tests for common slopes through a multivariate regression model (Mitteroecker et al., 2005; Cardini and Thorington, 2006). We used an analysis of covariance with the shape variables as dependent variables, populations as grouping variables, and centroid size as a covariate. This analysis allows estimating whether the variation in shape within two or more populations has the same relationship with size as the independent variable. Significant differences indicate that the groups being compared do

not share common allometries, and thus, the differences between them are not only the result of an extension or truncation of the same ontogenetic trajectory (Klingenberg, 1998; Mitteroecker et al., 2005). The multivariate regressions were done using tpsRegr 1.36 (Rohlf, 2009).

Comparison of Evolutionary and Ontogenetic Allometries

For comparisons between evolutionary and ontogenetic allometries, we calculated Pearson’s product-moment correlation coefficient between the regression vectors. Then, we calculated the arccosine of the correlation coefficients (i.e., the cosine of the angle) to obtain the angles between the regression vectors (Blackith and Reyment, 1971). The correlation values were compared to the distribution of values generated by bootstrap resampling ($n = 999$). The regression vector describing the evolutionary allometry was estimated from the OLS analysis of the consensus shape coordinates on the centroid size of each sample, while the vector describing the ontogenetic allometry was estimated using a pooled within-group regression of the four ontogenetic series (Pa, PG, ChV, SP-TF). Here we assumed that these four samples were representative of the range of variation among southern South American populations because they included the range of morphological and ecological variation in the region of study (see Ackermann and Cheverud, 2004 for a similar approach). The sample used to estimate the pooled within-group ontogenetic allometry included 55 subadults from 4 to 15 years old (Table 2) and 77 adults from the same four samples.

RESULTS

Evolutionary Allometry

The ordinations of population means calculated over the shape coordinates of adult crania are shown in Figure 3. The first two PCs explain more than 50% of total variation in the face, base and vault. The first PC mainly describes shape differences among subsistence groups for cranial vault and face.

Variation in cranial shape among populations was significantly related to size differences among them, although size accounted for less than 50% of shape variation. The multivariate regression of Procrustes coordinates of population means on centroid size accounted for 20%–40% of the total shape variation depending on the cranial unit analysed (Table 3). The highest percentage (39.86%) was obtained for facial shape, while for the vault and the base, the percentage was $\sim 20\%$. Similar results were achieved using the first and the first four PCs instead of the Procrustes shape coordinates (Table 3). Regression of PC1 (which accounted for 35%–45% of the total shape variation) on centroid size using PGLS indicated that the facial and vault shapes were significantly associated with size, while the shape of the cranial base showed a low and non-significant association with size. PGLS analysis of the first four PCs, which accounted for 80% of total variation, and centroid size showed that the shape variation in the cranial base was not significantly related to size, while the other cranial units exhibited a significant association (Table 3). The pattern of shape variation for evolutionary allometry estimated from the regression of the consensus shape of

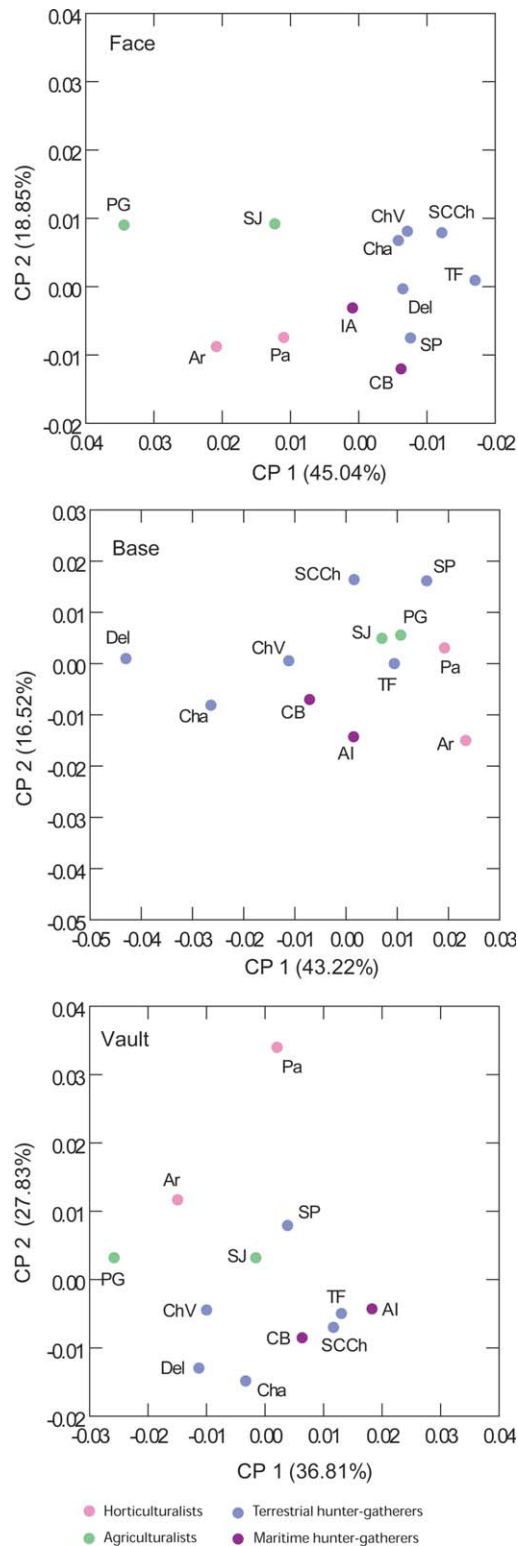


Fig. 3. Principal component (PC) analysis of cranial shape for the adult samples. Abbreviations are shown in Table 1.

each adult sample on centroid size indicated a trend towards a more robust and taller face and a longer vault with increasing size (Fig. 4).

TABLE 3. Evolutionary allometry. Regression of shape on centroid size (CS) between populations

PGLS regression model		Face	Vault	Base
PC1 versus CS	% predicted	85	39	14
	F	57.10 ^a	6.46 ^b	1.61
	PC1%	45.04	36.81	43.22
PCs1-4 versus CS	F	3.17 ^b	4.47 ^a	2.18
Procrustes shape coordinates versus CS	% predicted	39.86	24.15	19.54

Shape was extracted from the Procrustes shape coordinates, the first principal component (PC1) and the first four principal components that account for 80% of total variation (PCs1-4). PGLS: Phylogenetic Generalized Least-Squares model. % predicted: shape variation explained by size in the regression model.

^a $P < 0.01$

^b $P < 0.05$

Ontogenetic Allometry

Within each population, the multivariate regression of Procrustes shape coordinates on centroid size indicated that between 15% and 30% of the total shape variation in the face and vault is related to size during postnatal ontogeny (Table 4). For the cranial base the percentage of shape variation accounted by size was lower, ranging from 8% to 13% (Table 4). Regressions of PC1 and the first PCs accounting for 80% of shape variation on centroid size were significant for the three cranial units (Table 4). Thus, there was a significant association between shape and size in the ontogenetic series analyzed. It is interesting to note that size variation accounted for 50% to 70% of the shape variation along the first PC for the facial component (Table 4), while this percentage was considerably lower for the cranial base (from 4% to 40%, Table 4).

The slopes of the ontogenetic allometric trajectories were similar in the populations analyzed. The test of common slopes performed on the first 8 principal components, which accounted for 80% of sample variation, indicated that differences in slope among samples were not significant (Face: $F = 0.886$, $P > 0.01$; Vault: $F = 0.979$, $P > 0.01$; Base: $F = 1.448$, $P > 0.01$).

The multivariate pooled within-group regressions of the ontogenetic sample showed that size-related shape changes along ontogeny were similar to those observed for evolutionary allometry (Fig. 5). A clear trend towards a relatively taller and narrower face and a longer vault with increasing size was also observed (Fig. 5). The regressions of Procrustes coordinates on log centroid size indicated that size accounted for 18.48% of shape variation in the face, 5.16% in the vault and 9.21% in the base along the pooled ontogenetic sample.

Comparison of Evolutionary and Ontogenetic Allometries

The correlation between the regression vectors for evolutionary and ontogenetic (pooled within-group) allometries was high for the face ($r = 0.847$; 95.0% confidence

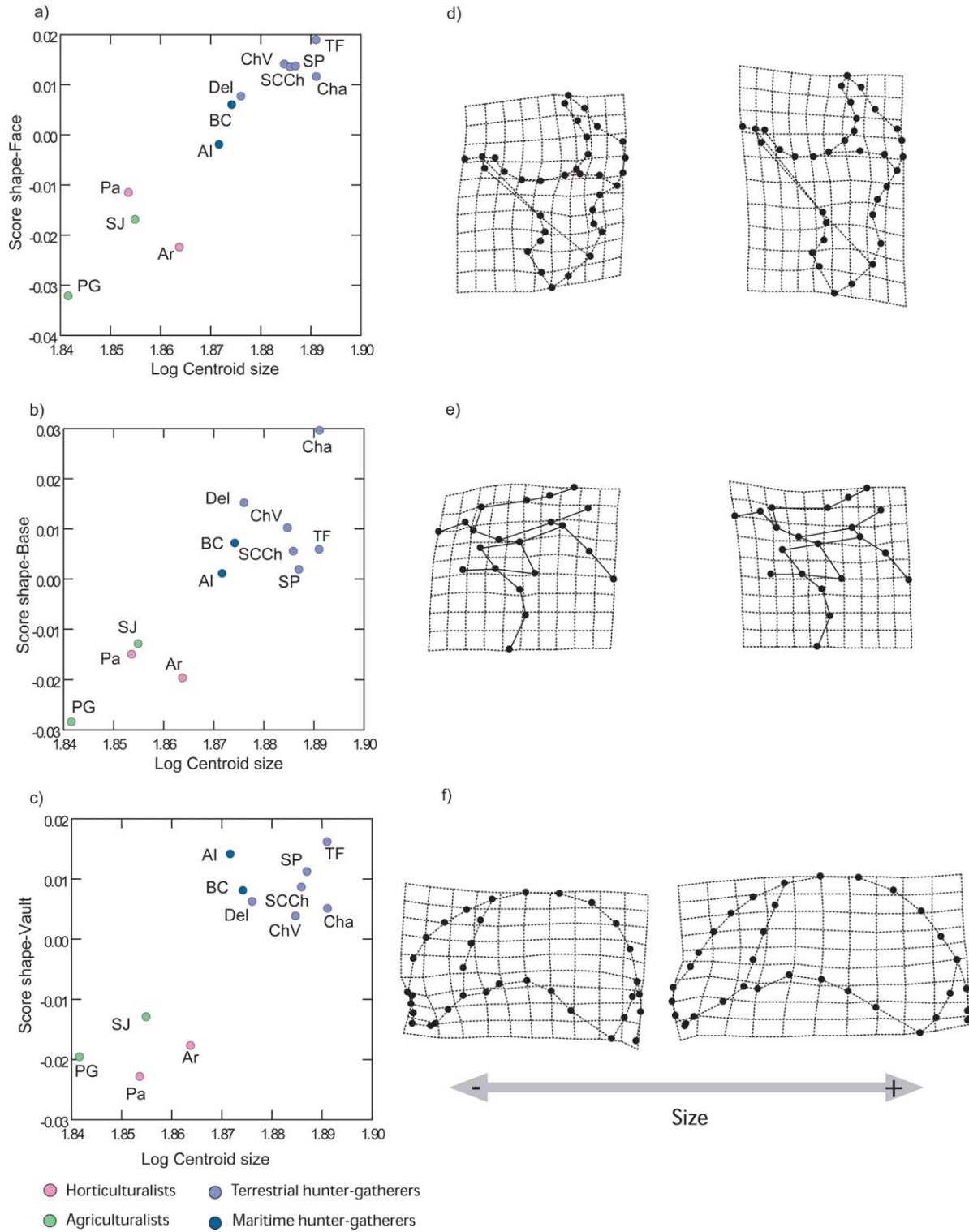


Fig. 4. Evolutionary allometry of South American human populations. (a–c) The shape scores, obtained from the multivariate regression of superimposed Procrustes coordinates on log centroid size, as a function of size. Deformation grids represent the shape changes associated with

evolutionary allometry, estimated from the regression of shape on log centroid size in the adult samples. Each pair of grids shows the deformation in shape from the reference to the lowest and the highest values of centroid size for the face (d), base (e), and vault (f).

TABLE 4. Ontogenetic allometry. Regression of shape on centroid size (CS) within populations

Component	Regression model		Ontogenetic series			
			Pa	ChV	PG	SP-TF
FACE	PC1 versus CS	% predicted	63	49	66	70
		F	67.99 ^a	34.77 ^a	40.49 ^a	61.86 ^a
	PCs (80%) versus CS	PC1%	34.12	35.24	21.36	30.44
		F	24.75 ^a	9.45 ^a	6.65 ^a	2.53 ^a
VAULT	Procrustes shape coordinates versus CS	% predicted	24.14	19.6	15.77	22.67
		F	61	72	38	15
	PC1 versus CS	% predicted	61.79 ^a	91.33 ^a	12.90 ^a	4.87 ^b
		PC1%	42.92	36.38	32.73	28.31
BASE	PCs (80%) versus CS	F	12.43 ^a	17.22 ^a	4.50 ^a	11.18 ^a
		% predicted	27.42	27.18	16.6	16.03
	Procrustes shape coordinates versus CS	% predicted	4	22	20	39
		F	1.48	10.26 ^a	5.18 ^b	17.60 ^a
	PC1 versus CS	PC1%	29.70	30.92	24.46	30.07
		F	14.71 ^a	8.64 ^a	2.18 ^b	5.14 ^a
	PCs (80%) versus CS	% predicted	10.62	11.72	7.86	13.2

Shape was extracted from the Procrustes shape coordinates, the first principal component (PC1), and the first principal components that account for 80% of total variation [PCs (80%)]. PC1%: percentage of total shape variation explained by the first principal component. % predicted: percentage of shape variation explained by size in the regression model. Abbreviations of sample names are shown in Table 1.

^a $P < 0.01$

^b $P < 0.05$.

interval = 0.762–0.905) and vault ($r = 0.651$; 95.0% confidence interval = 0.452–0.800). Conversely, the correlation between the regression vectors describing the evolutionary and ontogenetic allometries for the cranial base was low and non-significant ($r = 0.364$; 95.0% confidence interval = -0.010 – 0.640). The results obtained indicate that only the regression vectors for the cranial base are independent, exhibiting an angle of 68.65° , while the facial unit was characterized by the smallest angle (32.11°), followed by the vault (49.38°).

DISCUSSION

The human populations from southern South America diverged extensively in overall body and cranial size (Beguelin, 2009; Perez and Monteiro, 2009). Accordingly, our study showed that a significant amount of cranial shape disparity among these populations was due to allometric shape changes. This is particularly striking for the face and vault, for which between 20% and 40% of the shape variation represented by the Procrustes shape coordinates was explained by cranial size differences among samples. The PGLS analysis further confirmed the association between shape and size, independently of the shared evolutionary history among populations (Table 3).

The facial and vault shape changes associated with evolutionary allometry and ontogenetic allometry (Figs. 4 and 5) were characterized by a heightening of the face and a lengthening of the vault with increasing size. This indicates a common pattern of size-related shape changes, regardless of whether the increase in cranial size was due to factors affecting the mean size among populations or to ontogenetic growth within populations. The similarity between ontogenetic and evolutionary allometry, which was also supported by the common slope test, suggests that differences in the extent of growth could have greatly contributed to the cranial shape variation among human populations from the region under

study. According to these results, the processes that generate covariation among traits during individual growth could have channelled the shape variation of the face and vault related to size differences among populations (Klingenberg, 2005; Shingleton et al., 2007).

However, it is important to note that even though it is clear that the shape variation among South American populations was related to size, a large percentage of shape changes were independent of size and were probably related to other factors. This is evident from the regression results, which show that although the model explains a large amount of the shape variation among (20%–40%) and within (8%–30%) samples, there is a significant percentage of the original variation unaccounted for by size. Similar proportions of observed variation described by allometry have been obtained for other groups of organisms based on landmark coordinate data (Swiderski, 2003; Cardini and Thorington, 2006), in contrast to the dominant allometric pattern usually found in traditional distance measurements. The differences between the results obtained through these two approaches may be related to the limitation of traditional morphometrics to convey information about the geometric structure of morphological traits (Zelditch et al., 2004). This presents an important consequence for evolutionary studies, as was addressed by Swiderski (2003), who suggested that given the smaller amount of shape variation explained by allometric patterns, they might not be as important as was previously thought in directing evolutionary change.

A different pattern was found for the cranial base, for which size accounted for a smaller and non-significant percentage of the shape variation among populations (Table 3). Within ontogenetic series, the percentage of shape variation related to size was also lower than for the face and vault, although it was significant in the regression model between the principal components and centroid size (Table 4). Differences between cranial modules are expected because complex morphological

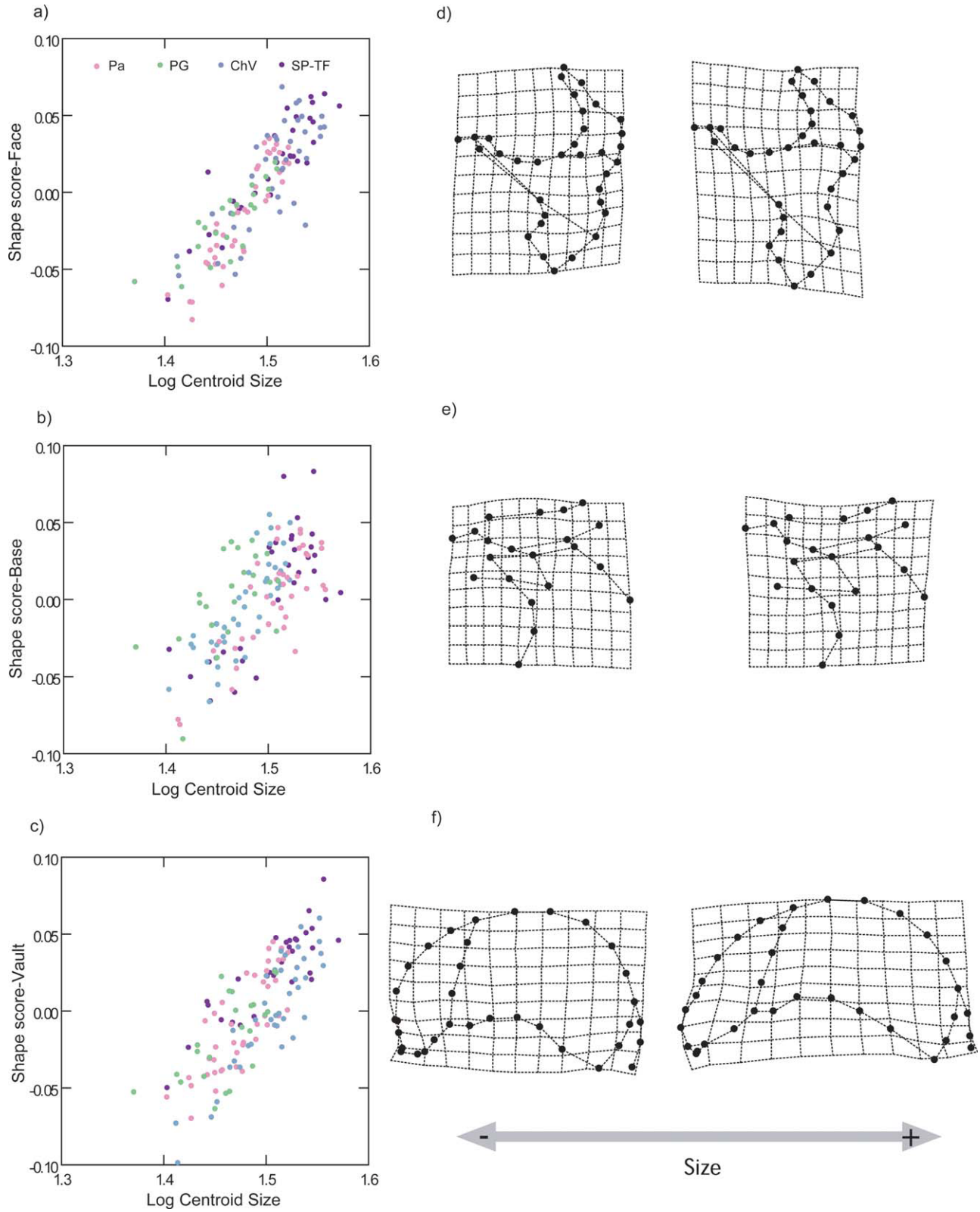


Fig. 5. Ontogenetic allometry of South American human populations. (a-c) The shape scores, obtained from the multivariate pooled within-group regression of superimposed Procrustes coordinates on log centroid size, as a function of size. Deformation grids represent the shape changes associated with ontogenetic allometry, estimated

from the regression of shape on log centroid size in the ontogenetic series. Each pair of grids shows the deformation in shape from the reference to the lowest and the highest values of centroid size for the face (d), base (e), and vault (f).

structures are not only controlled by common developmental processes, such as somatic growth, but also by local processes (Hallgrímsson and Lieberman, 2008; Mitteroecker and Bookstein, 2008). In this sense, the three cranial components differ in their embryological origin, mode of ossification and pattern of growth (Morris-Kay, 2001; McBratney-Owen et al., 2008). Particularly, the cranial base follows a growth pattern characterized by a rapid early size increase, whereas the facial skeleton follows the general pattern of somatic growth and attains its final size at an older age (Humphrey, 1998; Scheuer and Black, 2000; Sperber, 2001). The differences in timing among the modules can explain the discrepancies in the strength of the ontogenetic allometric pattern. The youngest specimens in the samples studied here are 4 years old, and thus a large amount of growth in the cranial base is not expected beyond that age, contrary to the face that continues to grow until at least 18 years of life. The discrepancy between the results obtained for the evolutionary allometry of the three modules of the cranium also indicates that they did not exhibit a uniform response to changes in overall size among populations. The large ecological variation displayed by the region under study might have had an important role driving the shape differentiation among populations (see discussion below), which in turn could have a greater effect on facial bones, more susceptible to differences among populations in the environment experienced during ontogeny due to their extended growth.

The results of this study seem to contrast those obtained in a previous study aimed at analysing the ontogenetic development of cranial robusticity in human populations from South America (Gonzalez et al., 2010). In that study we evaluated the ontogenetic allometric trajectories of a subset of cranial traits commonly used to compare the level of robusticity and found that their trajectories were not parallel among all samples, suggesting a divergence in size-related shape changes. Because we simultaneously analysed traits that correspond to different modules of the cranium, such a divergence in trajectories could result from a combination of ontogenetic scaling in the relatively independent units that integrate to make up this complex structure. Thus, it becomes evident that the selection of a particular set of morphometric variables will affect the patterns described and, therefore, the inferences made about the ontogenetic modifications responsible for morphological differentiation. In this sense, analyses of allometry should be performed taking into account the patterns of integration and modularity of the anatomical structures investigated. A further problem arising within this approach is the fact that it usually turns out to be difficult to determine the actual regions that possess a common evolution of ontogeny for all of their morphometric variables (Mitteroecker et al., 2005). This requires studies that combine data about the developmental basis and functional properties of such structures with morphometric techniques applied to the discovery and characterisation of modularity (Hallgrímsson et al., 2009; Perez et al., 2009). The division of the cranium into the three modules used here is well supported by different lines of evidence; though given the hierarchical structure of modularity, further subdivisions can be carried out based on different criteria.

In this study, we focused on the influence of size on the pattern of cranial shape variation among human

populations from South America and evaluated the type of alterations in the ontogenetic allometric trajectories that could account for the differences observed at the among-population level. We found that a significant amount of shape diversity in the face and vault among these populations was due to size-related changes and that the allometric change among populations followed the pattern of ontogenetic allometry. One question that remains to be discussed is about the factors driving such differences in size. Available studies suggest that size variation in the region under study could be related to disparities in the nutrient intake, in terms of quantity and quality, among groups with different subsistence strategies (Perez and Monteiro, 2009; Bernal et al., 2010; Perez et al., 2011). Nutritional status is one of the most important factors that regulate growth and, as a consequence, the overall adult body size (Nijhout, 2003). It is well known that developmental plasticity can play a major role in explaining morphological differentiation of ecologically diverse organisms (Pigliucci, 2001). Taken together our results indicate that plasticity of ontogenetic trajectories, in the form of extensions or truncations of growth trajectories, in response to ecological factors could have been an important source of morphological differentiation in different cranial structures as a result of size-related shape changes.

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LITERATURE CITED

- Ackermann RR, Cheverud, JM. 2004. Detecting genetic drift versus selection in human evolution. *Proc Natl Acad Sci USA* 101:17946–17951.
- Adams DC, Nistri A. 2010. Ontogenetic convergence and evolution of foot morphology in European cave salamanders (Family: Plethodontidae). *BMC Evol Biol* 10:216.
- Beguelin M. 2009. Variación geográfica en la morfología del esqueleto postcranial de las poblaciones humanas de Pampa y Patagonia durante el Holoceno tardío: una aproximación morfométrica. PhD Thesis. Facultad de Ciencias Naturales y Museo, Universidad Nacional de La Plata, La Plata.
- Bernal V, Perez SI, Gonzalez PN. 2006. Variation and causal factors of craniofacial robusticity in Patagonian hunter-gatherers from late Holocene. *Am J Hum Biol* 18:748–765.
- Bernal V, Perez SI, Gonzalez PN, Felizola Diniz-Filho JA. 2010. Ecological and evolutionary factors in dental morphological diversification among modern human populations from southern South America. *Proc R Soc B* 277:1107–1112.
- Blackith R, Reyment RA. 1971. Multivariate morphometrics. New York: Academic Press.
- Bookstein FL. 1991. Morphometric tools for landmark data: geometry and biology. Cambridge: Cambridge University Press.
- Bookstein FL, Streissguth AP, Sampson PD, Connor PD, Barr HM. 2002. Corpus callosum shape and neuropsychological deficits in adult males with heavy fetal alcohol exposure. *Neuroimage* 15:233–251.
- Cardini A, Thorington RW. 2006. Postnatal ontogeny of marmot (rodentia, Sciuridae) crania: allometric trajectories and species divergence. *J Mammal* 87:201–215.
- Cheverud JM. 1982. Phenotypic, genetic, and environmental morphological integration in the cranium. *Evolution* 36:499–516.

- Cobb SN, O'Higgins P. 2004. Hominins do not share a common post-natal facial ontogenetic shape trajectory. *J Exp Zool Mol Dev Evol* 302B:302–321.
- Drake AG, Klingenberg CP. 2008. The pace of morphological change: historical transformation of skull shape in St Bernard dogs. *Proc R Soc B* 275:71–76.
- Felsenstein J. 1985. Phylogenies and the comparative method. *Am Nat* 125:1–15.
- Frankino WA, Zwaan BJ, Stern DL, Brakefield PM. 2005. Natural selection and developmental constraints in the evolution of allometries. *Science* 307:718–720.
- Gerber S, Eble GJ, Neige P. 2008. Allometric space and allometric disparity: a developmental perspective in the macroevolutionary analysis of morphological disparity. *Evolution* 62:1450–1457.
- Goebel T, Waters MR, O'Rourke DH. 2008. The late pleistocene dispersal of modern humans in the Americas. *Science* 319:1497–1502.
- Gonzalez PN, Perez SI, Bernal V. 2010. Ontogeny of robusticity of craniofacial traits in modern humans: a study of south American populations. *Am J Phys Anthropol* 142:367–379.
- Gould, SJ. 1966. Allometry and size in ontogeny and phylogeny. *Biol Rev* 41:587–640 587.
- Gustafsson A, Lindenfors P. 2004. Human size evolution: no evolutionary allometric relationship between male and female stature. *J Hum Evol* 47:253–266.
- Hallgrímsson B, Jamniczky H, Young N, Rolian C, Parsons T, Boughner J, Marcucio R. 2009. Deciphering the palimpsest: studying the relationship between morphological integration and phenotypic covariation. *Evol Biol* 36:355–376.
- Hallgrímsson B, Lieberman DE. 2008. Mouse models and the evolutionary developmental biology of the skull. *Int Comp Biol* 48:373–384.
- Hendrikse JL, Parsons TE, Hallgrímsson B. 2007. Evolvability as the proper focus of evolutionary developmental biology. *Evol Dev* 9:393–401.
- Humphrey LT. 1998. Growth patterns in the modern human skeleton. *Am J Phys Anthropol* 105:57–72.
- Jamniczky HA, Boughner JC, Gonzalez PN, Powell CD, Rolian C, Schmidt EJ, Parsons TE, Bookstein FL, Hallgrímsson B. 2010. Mapping the epigenetic landscape: rediscovering Waddington in the post-genomic age. *BioEssays* 32:1–6.
- Klingenberg CP. 1996. Multivariate allometry. In: Marcus LF, Corti M, Loy A, Naylor GJP, Slice DE, editors. *Advances in morphometrics*. New York: Plenum Press. p23–49.
- Klingenberg CP. 1998. Heterochrony and allometry: the analysis of evolutionary change in ontogeny. *Biol Rev* 73:79–123.
- Klingenberg CP. 2005. Developmental constraints, modules and evolvability. In: Hallgrímsson B, Hall BK, editors. *Variation*. San Diego: Academic Press. p219–247.
- Klingenberg CP. 2010. There's something afoot in the evolution of ontogenies. *BMC Evol Biol* 10:221.
- Lande R. 1979. Quantitative genetic analysis of multivariate evolution, applied to brain: body size allometry. *Evolution* 33: 402–416.
- 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.
- Marroig M. 2007. When size makes a difference: allometry, life-history and morphological evolution of capuchins (*Cebus*) and squirrels (*Saimiri*) monkeys (Cebinae, Platyrrhini). *BMC Evol Biol* 7:20.
- McBratney-Owen B, Iseki S, Bamforth SD, Olsen BR, Morriss-Kay GM. 2008. Development and tissue origins of the mammalian cranial base. *Dev Biol* 322:121–132.
- Mitteroecker P, Bookstein FL. 2008. The evolutionary role of modularity and integration in the hominoid cranium. *Evolution* 62:943–958.
- Mitteroecker P, Gunz P, Bernhard M, Schaefer K, Bookstein FL. 2004. Comparison of cranial ontogenetic trajectories among hominoids. *J Hum Evol* 46:679–698.
- Mitteroecker P, Gunz P, Bookstein FL. 2005. Heterochrony and geometric morphometrics: a comparison of cranial growth in *Pan paniscus* versus *Pan troglodytes*. *Evol Dev* 7:244–258.
- Morriss-Kay GM. 2001. Derivation of the mammalian skull vault. *J Anat* 199:143–151.
- Nijhout HF. 2003. The control of growth. *Development* 130:5863–5867.
- Percival C, Chimera M, Kim M, Kenney-Hunt J, Conley A, O'Connor C, Roseman C, Cheverud J, Richtsmeier J. 2009. A study of 2D landmark data accuracy in representing 3D mouse skull form. *Am J Phys Anthropol* 138:209.
- Perez SI, Bernal V, Gonzalez PN. 2006. Differences between sliding semi-landmark methods in geometric morphometrics, with an application to human craniofacial and dental variation. *J Anat* 208:769–784.
- Perez SI, Bernal V, Gonzalez PN. 2007. Morphological differentiation of aboriginal human populations from Tierra del Fuego (Patagonia): implications for South American peopling. *Am J Phys Anthropol* 133:1067–1079.
- Perez SI, de Aguiar MAM, Guimarães PR, Jr, dos Reis SF. 2009. Searching for modular structure in complex phenotypes: Inferences from network theory. *Evol Biol* 36:416–422.
- Perez SI, Lema V, Diniz-Filho JAF, Bernal V, Gonzalez PN, Gobbo D, Pucciarelli HM. 2011. Geographic variation, diet and temperature in cranial morphology of South American human populations: an approach based on spatial regression and rate tests. *J Biogeogr* 38:148–163.
- Perez SI, Monteiro LR. 2009. Nonrandom factors in modern human morphological diversification: a study of craniofacial variation in southern South American populations. *Evolution* 63:978–993.
- Pigliucci M. 2001. Phenotypic plasticity: beyond nature and nurture. Baltimore: The Johns Hopkins University Press.
- Rohlf FJ. 2001. Comparative methods for the analysis of continuous variables: geometric interpretations. *Evolution* 55:2143–2160.
- Rohlf FJ. 2009. tps serie softwares. Available at <http://life.bio.sunysb.edu/morph/>.
- Rohlf FJ, Slice DE. 1990. Extensions of the Procrustes Method for the optimal superimposition of landmarks. *Syst Zool* 39:40–59.
- Scheuer L, Black S. 2000. Developmental juvenile osteology. San Diego: Academic Press.
- Sheets HD, Covino KM, Panasiwicz JM, Morris SR. 2006. Comparison of geometric morphometric outline methods in the discrimination of age-related differences in feather shape. *Front Zoo* 3:15.
- Shingleton AW, Frankino WA, Flatt T, Nijhout HF, Emlen DJ. 2007. Size and shape: the developmental regulation of static allometry in insects. *BioEssays* 29:536–548.
- Sperber GH. 2001. Craniofacial development. Hamilton: BC Decker Inc.
- Steele J, Politis G. 2009. AMS 14C dating of early human occupation of southern South America. *J Archaeol Sci* 36:419–429.
- Strand Vidarsdottir U, O'Higgins P, Stringer C. 2002. The development of regionally distinct facial morphologies: a geometric morphometric study of population-specific differences in the growth of the modern human facial skeleton. *J Anat* 201:211–229.
- Swiderski DL. 2003. Separating size from allometry: analysis of lower jaw morphology in the fox squirrel, *Sciurus niger*. *J Mammal* 84:861–876.
- Ubelaker D. 1989. Human skeletal remains. Washington DC: Taraxacum Press.
- Willson LAB, Sanchez Villagra MR. 2010. Diversity trends and their ontogenetic basis: an exploration of allometric disparity in rodents. *Proc R Soc B* 277:1227–1234.
- Zelditch ML, Sheets HD, Fink WL. 2000. Spatiotemporal reorganization of growth rates in the evolution of ontogeny. *Evolution* 54:1363–1371.
- Zelditch ML, Swiderski DL, Sheets HD, Fink WL. 2004. Geometric morphometric for biologists: a primer. Londres: Academic Press.