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The role of diet and temperature in shaping cranial diversification of South American human populations: an approach based on spatial regression and divergence rate tests

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ABSTRACT

Aim Understanding the importance of ecological factors in the origin and maintenance of patterns of phenotypic variation among populations, in an explicit geographical context, is one of the main goals of human biology, ecology and evolutionary biology. Here we study the ecological factors responsible for craniofacial variation among human populations from South America.

Location South America.

Methods We studied a dataset of 718 males from 40 South American populations, coming from groups that inhabited different geographical and ecological regions. Cranial size and shape variation were studied using 30 cranial measurements. We first used spatial correlograms and interpolated maps to address spatial patterns. We then regressed the shape (principal component scores) and size variables against ecology (mean annual temperature and diet) using multiple and multivariate spatial regression. Finally, the expected magnitudes of shape and size divergence under the influence of genetic drift and mutations alone were evaluated using neutral expectation for the divergence rate.

Results The spatial correlograms showed a cline affecting the entire South American distribution. Interpolated maps showed that size and allometric shape vary from south-east to north-west. Multiple and multivariate regression analyses suggested that diet has the largest and most significant effect on this pattern of size and allometric shape variation. Finally, the results of the divergence rate test suggested that random processes alone cannot account for the morphological divergence exhibited by cranial size and allometric shape scores among southernmost populations.

Main conclusions Correlograms, spatial regression and divergence rate analyses showed that although local factors (neutral processes or local environmental conditions) are important to explain spatial interpopulation differentiation in cranial characteristics among these populations, there is significant correlation of cranial size and allometric shape variation with diet. Gene flow among human populations, or local environmental conditions, could explain spatial variation mainly at smaller spatial scales, whereas the large-scale pattern of the South American dataset is mainly related to the high proportion of carbohydrates and low proportion of proteins consumed.

Keywords

Human biology, morphometric techniques, quantitative genetic models, shape differences, size variation, South America, spatial autocorrelation, spatial comparative techniques.

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INTRODUCTION

Understanding the importance of ecological factors in the origin and maintenance of regional and continental patterns of phenotypic variation among populations, in an explicit geographical context, is one of the main goals of human biology, ecology and evolutionary biology (Reznick *et al.*, 1997; Katzmarzyk & Leonard, 1998; Schluter, 2000; Roseman, 2004; Badyaev, 2005; Carroll *et al.*, 2007). The multiple environmental influences experienced by organisms can lead to phenotypic differences among populations through genetic differentiation, that is, ecological factors may act directly as a selective agent (Katzmarzyk & Leonard, 1998; Schluter, 2000; Diniz-Filho *et al.*, 2008). In addition, the range of phenotypes produced by a given genotype depends on the environment in which the organism develops. This is because developmental, physiological and metabolic processes are normally sensitive to environmental variables such as temperature, population density, elevation and nutrients (Nijhout, 2003). This influence of the environment during ontogeny might be especially strong on the patterns of phenotypic variation observed at an intraspecific level – ecophenotypic variation within and among populations (Pucciarelli & Oyhenart, 1987; Bogin & Rios, 2003; Carroll *et al.*, 2007).

Diet and temperature are two ecological factors that play an important role in shaping morphological variation among human populations (Katzmarzyk & Leonard, 1998; Bogin & Rios, 2003). Several studies at global and regional scales have stressed the primary role of temperature, given that variables such as body mass, surface area/mass ratio, stature, and cranial size and shape are all correlated with temperature (Roberts, 1953; Beals *et al.*, 1984; Katzmarzyk & Leonard, 1998; Roseman, 2004; Bernal *et al.*, 2006; Beguelin, 2009; Gustafsson & Lindenfors, 2009). These studies have suggested that modern humans follow Bergmann's ecogeographical rule (Bergmann, 1847; Rensch, 1938), which states that the individuals from populations inhabiting colder regions are larger, due to adaptive processes that resulted in a lower surface area/mass ratio than in warmer regions (Katzmarzyk & Leonard, 1998).

However, it is clear that ecogeographical rules must be evaluated within a more complex framework that takes into account multiple ecological and evolutionary factors that may drive morphological variation (Guillaumet *et al.*, 2008; Diniz-Filho *et al.*, 2009a). Indeed, in the context of human morphological variation, different analyses have shown that diet and body size are correlated, suggesting that diet may be an explanatory variable for size (Bogin & Rios, 2003). In particular, nutritional deterioration during growth seems to result in decrease of body mass, stature and cranial size of farmer groups (Larsen, 2006; Stynder *et al.*, 2007; Perez & Monteiro, 2009). However, the independent contribution of diet and temperature to morphological variation is difficult to assess in several geographical regions where temperature is associated with variation in subsistence or diet

(e.g. Beals *et al.*, 1984; Katzmarzyk & Leonard, 1998; Bernal *et al.*, 2006; Perez & Monteiro, 2009). This is because in tropical and subtropical regions, important changes in subsistence strategies occurred around 9000–4000 years ago with the advent of agricultural practices, whereas hunter-gatherer practices have persisted until recent times in temperate and cold regions (Harlan, 1971; Larsen, 2006; Price, 2009).

Here we study the ecological factors responsible for cranial variation among human populations from South America, a region with particular characteristics that make it a unique setting in which to analyse the relative importance of ecological factors on human diversification. This region is characterized by large environmental variation, mainly in available diet and temperature, and, most importantly, these variables are not directly associated. Although it was the last continent to be colonized by modern humans (*c.* 12,500–13,000 years ago; Borrero, 1999; Steele & Politis, 2009), South America displays exceptionally high levels of morphological variation among human populations (González-José *et al.*, 2005; Bernal *et al.*, 2006; Perez *et al.*, 2007a; Perez & Monteiro, 2009), particularly when compared with geographically larger regions around the world (Sardi *et al.*, 2005). In addition, molecular studies suggest that these populations have a recent and single origin (Moraga *et al.*, 2000; Fagundes *et al.*, 2008; Goebel *et al.*, 2008), thus the large morphological variation observed would result from the influence of local factors within the small time-scale of the peopling of the continent. Previous studies have related the morphological variation of South American populations to either temperature, through the action of natural selection (Rothhammer & Silva, 1990), or subsistence or diet, as a consequence of a developmental response during ontogeny (González-José *et al.*, 2005; Perez & Monteiro, 2009). However, no exhaustive evaluation of such hypotheses has been performed to date, especially in an explicit geographical context.

The goal of this study is to evaluate the importance of diet and temperature in shaping the craniofacial variation among human populations from South America. Craniofacial morphology is quantified through linear measurements that are specifically used to describe the functional components of the skull (Pucciarelli *et al.*, 2006). We test the correspondence of cranial size and shape variation with dietary composition and mean annual temperature, using spatial regression techniques that take into account the spatial structure of samples (Dormann *et al.*, 2007; Diniz-Filho *et al.*, 2009b; Perez *et al.*, 2010). Subsequently, we test the hypothesis that cranial diversification among South American populations was the product of random processes alone, using a hierarchical multigroup comparison approach that calculates divergence rate values at multiple spatial scales (Lynch, 1990; Ackermann & Cheverud, 2002; Stepan *et al.*, 2002). We would reject the null hypothesis of random processes if there is an association between ecological factors and morphological variation in a geographical context.

MATERIALS AND METHODS

Samples

We studied a dataset of 718 males from 40 South American populations (Fig. 1; Pucciarelli *et al.*, 2006). All samples correspond to recent human populations (Table 1) and come from groups that inhabited different geographical and ecological regions. Distribution of samples ranges from 12° N latitude to 54° S latitude, and mean annual temperature in the sampled area ranges from 28 to 8 °C (Fig. 2). Samples comprise farming groups (groups with agricultural economy); horticulturalists (groups with a small-scale, highly diversified cultivation system); pastoralists; terrestrial hunter-gatherers; and maritime hunter-gatherers (Harlan, 1971; Harris, 1989; Pearsall, 1992, 2008; Berberian & Nielsen, 2001; Erickson, 2008). Geographical provenance of the samples was obtained from collection databases. The geographical coordinates of each local population were transformed to a geodesic system (decimal degrees of latitude and longitude) and used to

compute a matrix of great circle geographical distances between population pairs.

Data on mean annual temperature and dietary composition for each local population were collected to be used as estimators of environmental variation across the subcontinent (Beals *et al.*, 1984; Katzmarzyk & Leonard, 1998), considering that these factors may have shaped the morphological divergence among South American human populations (Rothhammer & Silva, 1990; Bernal *et al.*, 2006; Perez & Monteiro, 2009). A variable to account for differences in hardness of diet, such as the presence of pottery and grinding stone tools for food preparation, was not considered because during the late Holocene these artefacts were widespread in every geographical region analysed (Silverman & Isbell, 2008; Bernal *et al.*, 2010).

Temperature was used as an indirect estimator of climate (Fig. 2), and data were obtained for each of the 40 populations (for each geographical site or closest available location) from the WorldClim dataset (representative of 1950–2000; <http://www.worldclim.org/current>, accessed May 2009).

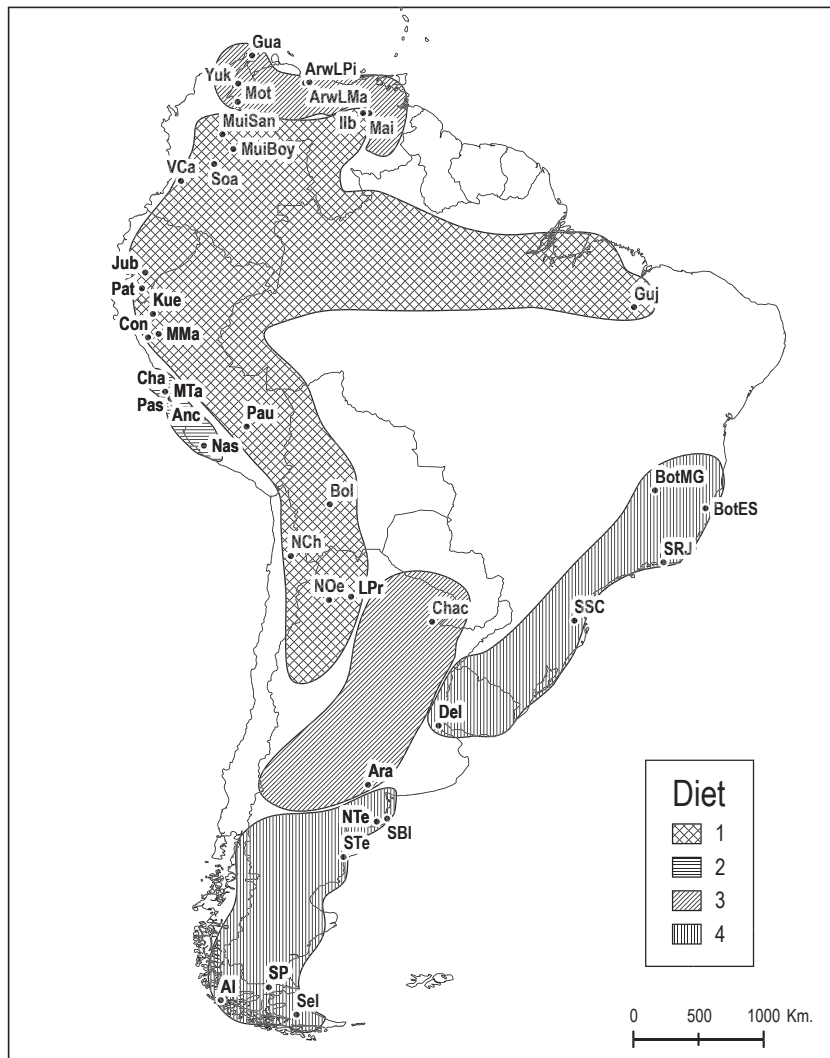


Figure 1 Map showing the central geographical location of the 40 South American human populations sampled. Locality names are defined in Table 1. Diet is also plotted onto the map: (1) carbohydrate-rich diet; (2) carbohydrate-rich diet with minor marine protein component (molluscs and fishes); (3) diet based mainly on terrestrial proteins with a minor carbohydrate component; (4) protein-rich diet. Projection: Geographic. Datum: WGS84.

Table 1 The 40 localities from which the South American human populations were sampled, together with their abbreviations, sample sizes (*n*) and geography-based South American hierarchies, used to calculate Δ divergence rates (Lynch, 1990).

Locality	Abbrev.	<i>n</i>	Hierarchy 1	Hierarchy 2	
Motilon	Mot	20	North	Venezuela and Colombia	
Guajiro	Gua	14			
Yukpa	Yuk	13			
Arawak	ArwLPi	14			
Arawak	ArwLMa	13			
Maipure	Mai	15			
Ipi-Iboto	Iib	17			
Muisca	MuiSan	12			
Muisca	MuiBoy	12			
Soacha	Soa	14			
Valle Cauca	VCa	12			
Calabaso	Jub	17			
Paltacalo	Pat	29			Ecuador and Peru
Kuelap	Kue	10			
Contumaza	Com	12			
Maqui-Maquis	MMA	10			
Chancay	Cha	16			
Pasamayo	Pás	11			
Makat Tampu	MTa	20			
Ancon	Anc	26			
Nasça	Nas	29			
Paucarcancha	Pau	11			
Guajajara	Guj	12	South	Southern Brazil and Chaco	
Botocudo	BotMG	10			
Botocudo	BotES	10			
Sambaqui	SRJ	12			
Sambaqui	SSC	22			
Noreste	Chac	36			
Deltaico	Del	27			
Boliviano	Bol	17			
Norchile	NCh	14			North-west Argentina, Bolivia and Chile
Noroeste	NOe	14			
Las Pirguas	LPr	15			
Araucano	Ara	17	Patagonia		
San Blas	SBl	12			
Nortehuelche	NTe	36			
Sudthuelche	STe	55			
Aonikenk	SP	11			
Selknam	Sel	34			
Yaghan	AI	17			

Data concerning diet were taken from specific archaeological and ethnohistorical literature sources for each human group (Muñoz Ovalle, 1989; Pearsall, 1992, 2003, 2008; Arellano, 1997; Herrera *et al.*, 1997; Segovia *et al.*, 2000; Scheinsohn, 2003; Polo Acuña, 2005; Bray, 2008; Drennan, 2008; Dulce Gaspar *et al.*, 2008; Guffroy, 2008; Michel López, 2008; Navarrete, 2008; Valdez, 2008; Zeidler, 2008; Restrepo, 2009; Scheel-Ybert *et al.*, 2009). The available information was classified according to dietary components (carbohydrates from domesticated vegetables versus protein from terrestrial mammalian or marine molluscs; Larsen, 2006). Two classi-

fication schemes were created for dietary data. In the first classification scheme (A), the samples were divided into four major groups: (1) carbohydrate-rich diet; (2) carbohydrate-rich diet with a minor component of marine protein (molluscs and fish); (3) diet based mainly on terrestrial proteins with a minor carbohydrate component; and (4) protein-rich diet (Fig. 1). A diet was classified as 'rich' in a given component when the said component was available and consumed all year round, while the term 'minor' is used when the component was consumed only seasonally. In the second classification scheme (B), the two carbohydrate-rich groups (diets 1 and 2) and the two protein-rich groups (diets 3 and 4) were clustered to obtain two larger groups used for multiple and multivariate regression analyses in which diet and temperature were independent variables. The classification schemes were binary-coded as dummy variables for statistical analysis. This coding allows the use of the classification schemes in the regression analyses (Legendre & Legendre, 1998).

Morphometric analyses

Cranial size and shape variation were studied using 30 cranial measurements (Pucciarelli *et al.*, 2006; see Appendix S1 in Supporting Information). All measurements were collected by one of us (H.M.P.) to avoid inter-observer error. Rather than performing a separate analysis on each of the 30 cranial variables, we used the original variables to calculate a size variable (geometric mean, GM; Jungers *et al.*, 1995) and principal components (PC) of the shape variables using the mean values of the sample.

The GM of all cranial measurements of each sample (a Mosimann size variable; Mosimann, 1970; Jungers *et al.*, 1995) was used as an overall craniofacial size measure. The GM was computed as the *n*th root of the product of the *n* variables (Jungers *et al.*, 1995; Pucciarelli *et al.*, 2006). Finally, we performed a principal components analysis (PCA; covariance matrix) using Mosimann shape variables (ratio; Jungers *et al.*, 1995). The Mosimann shape variables were calculated by dividing each original variable by the GM of all variables (Jungers *et al.*, 1995). The PC scores were calculated using singular value decomposition. This procedure results in data reduction and avoids redundancy (Marcus, 1990; Relethford, 2008). In addition, PC scores can be interpreted as low dimensional axes of Euclidean distance space.

Statistical analyses

To investigate the factors responsible for cranial variation among South American populations, we (1) explored the spatial structure of morphometric data, (2) analysed the correspondence between morphometric variation and environmental variables, and (3) tested the magnitude of morphometric divergence against the expected divergence values under the influence of genetic drift and mutations alone.



Figure 2 Spatial patterns of mean annual temperature in South America expressed in °C. Temperature was obtained from the WorldClim dataset, representative of 1950–2000 (<http://www.worldclim.org/> current, accessed May 2009).

Spatial autocorrelation techniques

Initially, spatial correlograms were generated to explore the spatial autocorrelation in size (GM) and shape (PC) variation. Although alternative approaches have been proposed to describe spatial patterns (e.g. variograms; Relethford, 2008), correlograms have been used successfully in previous exploratory autocorrelation analyses of human interpopulation variation, based mainly on genetic data (e.g. Sokal & Oden, 1978; Sokal *et al.*, 1989; Barbujani, 2000). The magnitude of autocorrelation was evaluated using Moran's *I* autocorrelation coefficient, which is given by:

$$I = \left(\frac{n}{S} \right) \left[\frac{\sum_i \sum_j (y_i - \bar{y})(y_j - \bar{y}) w_{ij}}{\sum_i (y_i - \bar{y})^2} \right]$$

where n is the number of local populations, y_i and y_j are the values of the morphological trait measured in the populations i and j , \bar{y} is the mean y , and w_{ij} is an element of the **W** matrix. In this **W** matrix, the elements are equal to 1 if the pair i, j of local populations is within the class interval of a given distance (indicating that samples in this class are 'connected'); otherwise $w_{ij} = 0$. S is the number of entries

(connections) in the **W** matrix. The value expected under the null hypothesis (no spatial autocorrelation) is $-1/(n - 1)$. Moran's *I* is usually calculated using several distance classes, and in this case multiple **W** matrices were built by connecting pairs of local populations separated by increasing geographical distances. This sequence of coefficients was plotted against geographical distances, generating a spatial correlogram (Sokal & Oden, 1978; Legendre & Legendre, 1998). Here, Moran's *I* coefficients were calculated for nine geographical distance classes, whose intervals were defined so that each class contained approximately the same number of connections among local populations (86 connections). Statistical significance of Moran's *I* autocorrelation coefficients was calculated using 4999 randomizations (for details see Legendre & Legendre, 1998).

In addition, the univariate size (GM) and shape (PC1) variables were plotted against geographical position using interpolated maps (Legendre, 1993; Legendre & Legendre, 1998). We estimated the unknown value of the size and shape variables for a given geographical location on a map of South America by local interpolation, using the observations available for neighbouring areas and the inverse distance weighting approach (Legendre & Legendre, 1998). First, we drew a

'search circle' around each unknown value, which was determined using five neighbour known values and a minimum of two neighbour known values that must be included in the interpolation for each unknown value. To each unknown value was assigned the value of the weighted mean of the known observations within the circle; $y_{\text{unknown}} = \sum w_i y_i$, where y_i is a known value and weight w_i is the inverse of the distance between the known value and the unknown value to be estimated (see below). This approach works under the assumption that the interpolated values are likely to resemble known values of the same area, and therefore the value of a known point used to calculate an unknown one is weighted in inverse proportion to the distance (i.e. the square root of the distance) between both points. This simple and robust approach has been widely applied in the natural sciences (Legendre, 1993).

Spatial regression techniques

To test the correspondence between morphometric variation and environmental variables, we regressed the shape (PC scores) and size (GM) variables against mean annual temperature and diet using multiple and multivariate spatial regression. We applied the generalized least-squares model (GLS), which is the same as the ordinary least-squares regression ($\mathbf{Y} = \mathbf{XB} + \varepsilon$, where \mathbf{Y} is the matrix describing the morphometric variation, \mathbf{X} is the matrix of independent variables, \mathbf{B} is the vector of regression coefficients, and ε is the error term), but incorporates autocorrelation into the residuals (ε). Therefore this model does not assume that the residuals are normally distributed with constant variance or are independently distributed among observations or populations (Dormann *et al.*, 2007; Diniz-Filho *et al.*, 2009b; Perez *et al.*, 2010). In the spatial regression technique, the error structure in the covariance matrix \mathbf{C} – which designs the covariance structure in ε – among residuals is designed to incorporate the expected lack of independence of the observations due to the spatial distribution of populations (i.e. $\mathbf{C} \neq \sigma^2 \mathbf{I}$; Perez *et al.*, 2010). Therefore in this model the covariance matrix \mathbf{C} is based on a matrix \mathbf{W} , the 'expected relationship matrix' or weighting matrix, which contains the correlation structure among the populations. The elements of \mathbf{W} were estimated by the inverse functions of great circle geographical distances (d_{ij}) between populations, $w_{ij} = \frac{1}{d_{ij}^2}$, which resulted in a large decline in distance with geographical distances ranging between 0 and 2000 km, whereas distances greater than c. 2500 km showed a plateau with little change in distance. We estimated R^2 and the standardized regression slopes of the spatial regression models, and assessed their significance using the t -statistic. Finally, we used Moran's I correlograms to test the assumption of spatial independence between the residuals of the spatial regression.

Because the environmental factors used as independent variables can present multicollinearity, we tested the association between mean annual temperature and diet (variable B) using Spearman's rank order correlation. The presence of multicollinearity could lead to misinterpretation of the

importance of these environmental variables for size and shape variation among South American human populations. However, Spearman's correlation (r_s) across the environmental variables was 0.26 ($P = 0.159$), suggesting absence of strong multicollinearity that could affect our results.

Divergence rate test

The expected divergence of shape (PC scores) and size (GM) under the influence of genetic drift and mutations alone were evaluated using Lynch's (1990) neutral expectation for the Δ divergence rate. We used this technique because of its simplicity and good performance when compared with other quantitative genetic models available (Perez & Monteiro, 2009). The Δ divergence rate observed (Lynch, 1990) is compared with the value of divergence expected based on the literature, in order to evaluate whether the amount of divergence is lower or higher than expected if mutation and random genetic drift were the only evolutionary forces acting (Lynch, 1990).

In this model, the rate of divergence is calculated as $\Delta = \text{var}_B(\ln z) / [t \text{var}_w(\ln z)]$, where $\text{var}_B(\ln z)$ and $\text{var}_w(\ln z)$ are the among- and within-population mean squares values calculated by an ANOVA, using the size (log-transformed) and shape scores as dependent variables, and population membership as a grouping factor. The overall GM was used as a size vector. To obtain the shape vector, we calculated PC scores based on the pooled within-group covariance matrices of ratio variables. The use of a subspace based on the pooled within-group covariance matrix is a conservative approach to analyse random factors, because this subspace does not preferentially consist of directions of variation for which among-group variance is high (Perez & Monteiro, 2009). In Lynch's model, t is the maximum number of generations since divergence, and is calculated by adding up the divergence times along each lineage. Lynch (1990) estimated that the lower and upper limits for divergence rates of mammalian skeletal traits under the neutral mutation-drift hypothesis were $\Delta_{\min} = 0.0001$ and $\Delta_{\max} = 0.01$, respectively. A range of divergence times was used to assess the duration of separation between the populations necessary for the observed Δ values to fall within the expected interval. Evidence of settlements as old as c. 13,000 years ago has been found in South America (Borrero, 1999; Steele & Politis, 2009). Fenner (2005) estimates the human generation time as 28 years. Thus we could set a conservative estimate of maximum $t = 928$ generations $[(13,000/28) \times 2]$ for the divergence of South American populations. The Δ divergence rate test is useful to detect directional non-random changes among recently separated populations (Hendry & Kinnison, 1999; Perez & Monteiro, 2009). After longer periods, stabilization of the divergence will erase all evidence of random or directional non-random factors occurring during the initial phase of divergence (Lemos *et al.*, 2001).

In order to avoid autocorrelation among samples (i.e. spatial structure), we used a spatial comparative approach, hierarchical multigroup comparison, to calculate Δ values (Ackermann & Cheverud, 2002; Stepan *et al.*, 2002). First, we calculated

the Δ divergence rate for all samples ($n = 40$). Next, the sample was divided into two geographical regions (North and South; Table 1) and the Δ value was calculated for each one. Finally, the North region was once more divided into two subregions (Venezuela and Colombia; Ecuador and Peru), while the South region was divided into three subregions (Bolivia, northern Chile and north-western Argentina; Patagonia; southern Brazil and Chaco; Table 1).

Statistical analyses were performed using SAM (Spatial Analysis in Macroecology) software version 3.1 (Rangel *et al.*, 2006) and R software 2.9.1 (R Development Core Team, 2009), which are freely available at <http://www.ecoevol.ufg.br/sam> and <http://www.r-project.org>, respectively. Interpolated Maps were obtained using GRASS software, which is freely available at <http://grass.itc.it/download/index.php>.

RESULTS

Spatial structure

The spatial correlograms showed a cline affecting the entire South American distribution, characterized by positive autocorrelation at short distances coupled with negative autocorrelation at large distances, for both the GM (for the 30 craniometric variables) and the scores in the first principal component of ratio variables (PC1; 44.6% of total variation; Fig. 3). The cline could be explained by several processes, such as directional gene flow among populations or ecological factors acting in geographically close and ecologically similar environments. The most influential variables along the PC1 score were mainly variables related to cranial width [neurocranial width (NW, Eurion-Eurion); masticatory height (MH, distance from the stephanion to the lower point of the zygotemporal suture); posteroneural width (PNW, Asterion-Asterion); anteroneural width (ANW, Pterion-Pterion); facial width (FW, Zygion-Zygion); and neurocranial length (NL, Nasion-Opisthocranium), all positive]. In addition, the PC1 score is strongly correlated with the variable GM [$r = 0.837$, $P = 0.005$; P value corrected for spatial autocorrelation using the method of Dutilleul (1993)]. PC2 (21.3%) and PC3 (15%) did not show spatial structure (results not shown). The most important variables along the PC2 score were related to cranial height [midneural height (MNH, Basion-Bregma); posteroneural width (PNW, Asterion-Asterion); neurocranial height (NH, Basion-Vertex), all positive] and cranial length [neurocranial length (NL, Nasion-Opisthocranium); anteroneural length (ANL, Glabella-Bregma); and posteroneural length (PNL, Opistion-Opisthocranium), all negative]. Finally, the most important variables along the PC3 score were cranial length variables [midneural length (MNL, Bregma-Lambda); posteroneural length (PNL, Opistion-Opisthocranium); and neurocranial length (NL, Nasion-Opisthocranium), all negative].

The patterns of size and PC1 shape variation did not show simple latitudinal trends and were not related to temperature variation. They vary from south-east to north-west, with mean

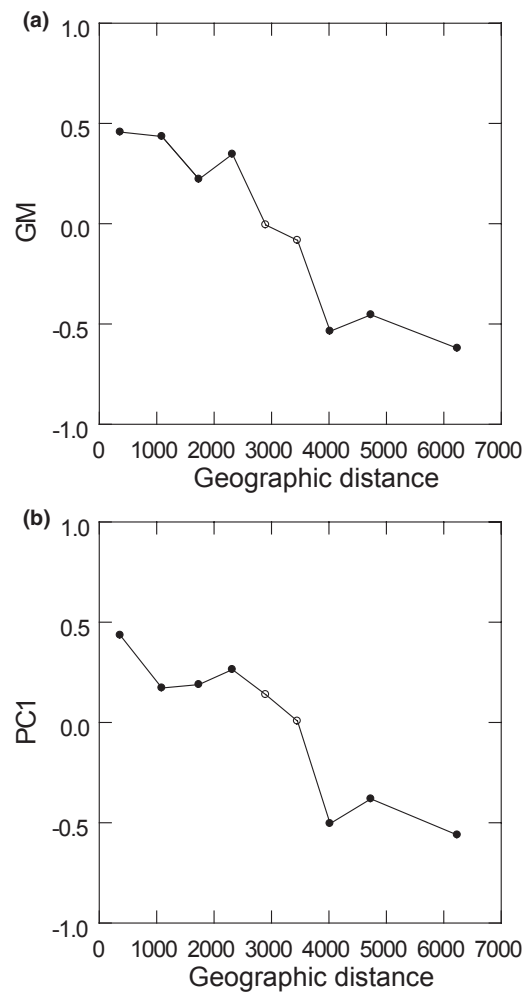


Figure 3 Autocorrelograms of size and shape variables for the 40 South American human populations studied: (a) for the geometric mean (GM) of cranial morphometric variables; (b) for the first principal component (PC1) calculated over the mean values of the Mosimann shape variables (ratio). Filled circles, significant autocorrelation coefficient values. Geographical distances (km) were calculated between the central locations of each population sampled.

sizes tending to be larger in the former (Fig. 4a). This pattern is apparently related to dietary differences among populations (Fig. 1). Mean cranial size was greater among groups from Pampa, Patagonia and southern Brazil, and smaller in groups from Peru, Ecuador and north-eastern Brazil. Some groups with intermediate cranial size occurred in Colombia and Venezuela. The PC1 (the allometric score; Fig. 4b) showed a similar variation pattern. Crania were wider among groups from Peru, Ecuador and north-eastern Brazil, and narrower in groups from Pampa, Patagonia and southern Brazil.

Spatial regression

The multiple and multivariate regression analyses suggested that diet has the largest and most significant ($P < 0.001$) effect

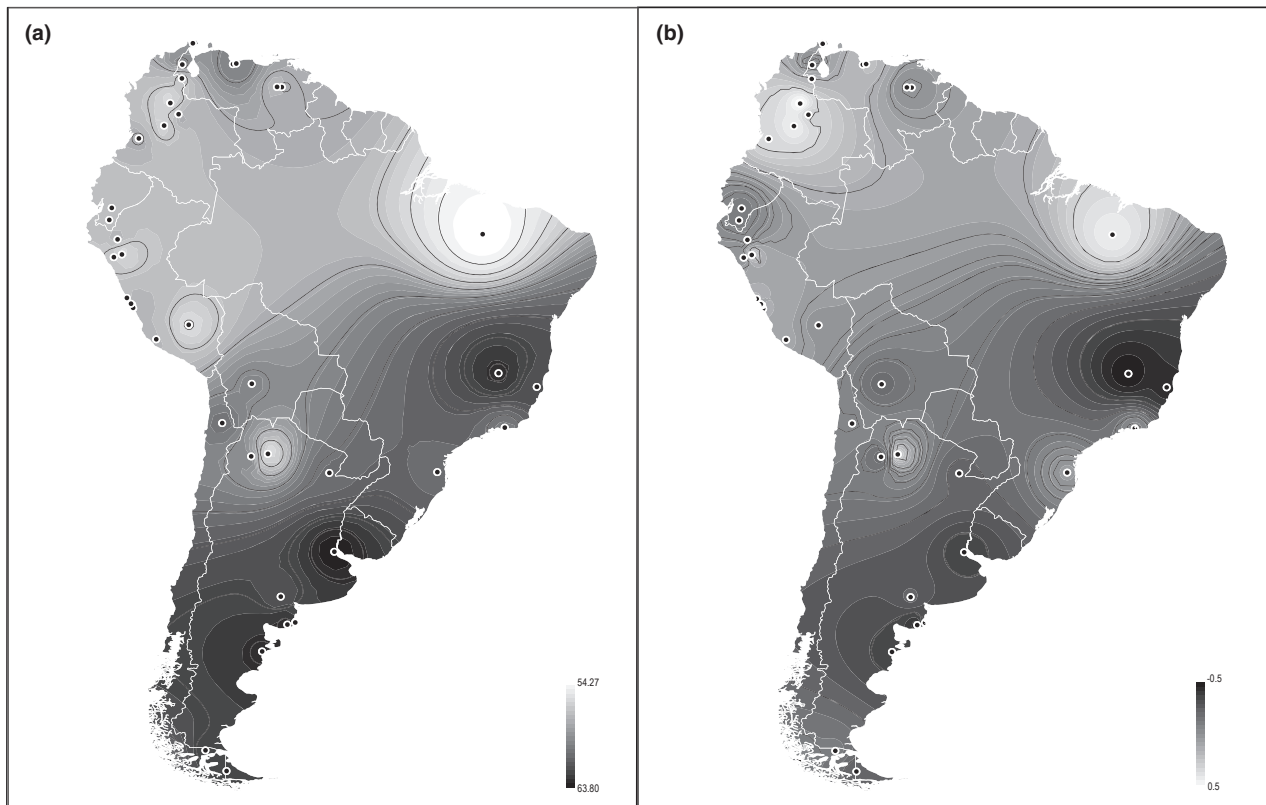


Figure 4 Geographical patterns of (a) the geometric mean (GM) of cranial morphometric variables; (b) the first principal component (PC1) calculated over the mean values of the Mosimann shape variables (ratio). The plotted map was created for the 40 South American human populations studied using Interpolated Maps with the inverse distance-weighting approach. Interpolated Maps were obtained using GRASS software, freely available at <http://grass.itc.it/download/index.php>.

on patterns of size and allometric shape variation (Tables 2 & 3; Fig. 5). Table 2 shows the results from GLS using diet (variable B) and temperature as predictor variables, and GM and PC1 as dependent variables, where the slope value was highest for diet and small for temperature. The size variable showed clear association with diet, with the largest crania occurring among hunter-gatherer groups (diet 4). In general, qualitative results were the same for the allometric shape variable (PC1): the most important factor driving cranial variation is diet, with partial standardized slopes of -0.526 . However, the results were different for the PC2 and PC3 variables, which are not associated with diet or temperature. Table 3 shows the results of GLS using only diet (variable A) as a predictor variable. Diet explained 42% and 68% of size and allometric shape variation, respectively (Table 3). The multiple and multivariate regression analyses of size and shape variation using only temperature as a predictor variable suggested that this variable did not have a significant effect (result not shown).

Divergence rate test

The results of the Δ divergence rate test suggested that random processes alone cannot account for the morphological diver-

Table 2 Multiple and multivariate regression of the environmental variables [mean annual temperature and diet, grouping diets 1 + 2 and 3 + 4 (variable B)] on size and shape variation for the whole skull, taking into account lack of independence resulting from geographical location.

	<i>F</i>	<i>R</i> ²	Variable B	
			Mean temperature	Diet
GM	22.652**	0.550	-0.223	-0.667**
PC1 (44.60%)	8.251**	0.308	-0.185	-0.526*
PC2 (21.34%)	0.771	0.040	-0.095	0.075
PC3 (14.97%)	4.489	0.195	0.319	-0.236
PC1–3 (80.91%)	3.083*	–	–	–

Shape is described using the first, second and third principal component scores (PC1, PC2, PC3) and the first three PCs together (PC1–3). Size is described using the geometric mean (GM) for all cranial measurements. Results were obtained for all 40 South American human populations studied.

* $P > 0.01$; ** $P > 0.001$.

gence among populations shown by cranial size (GM) and allometric shape (PC1) scores (Table 4; Fig. 6). The Δ values for these variables were consistently larger than $\Delta_{\max} = 0.01$

Table 3 Multiple and multivariate regression of diet (variable A, binary-coded as dummy variables for these statistical analyses) on size and shape variation for the whole skull, taking into account the lack of independence due to geographical location.

	F	R ²
GM	25.379**	0.679
PC1 (44.60%)	8.612**	0.418
PC2 (21.34%)	2.206	0.155
PC3 (14.97%)	3.135	0.207
PC1–3 (80.91%)	4.725**	–

Shape is described using the first, second and third principal component score (PC1, PC2, PC3) and the first three PCs together (PC1–3). Size is described using the geometric mean (GM) for all cranial measurements. Results were obtained for all 40 South American human populations studied.

P* > 0.01; *P* > 0.001.

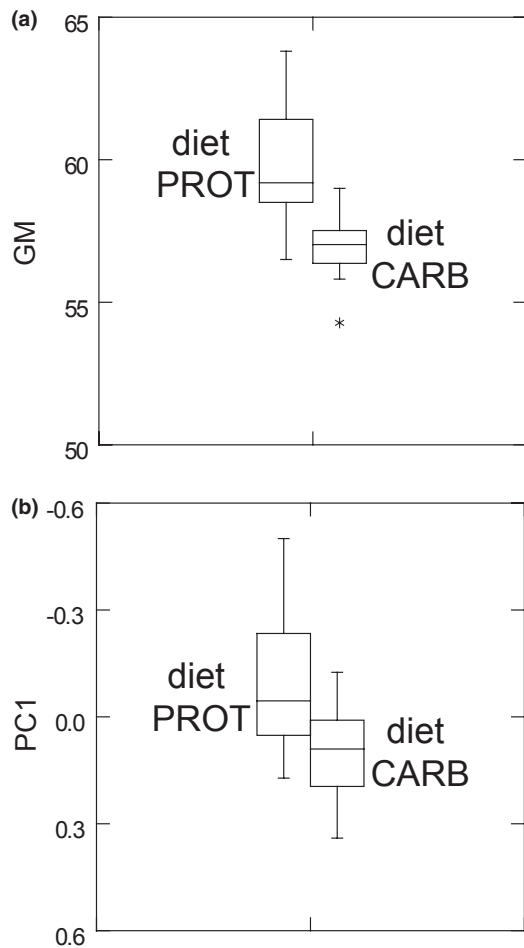


Figure 5 Box plot depicting the patterns of variation in (a) the geometric mean (GM) of cranial morphometric variables; (b) the first principal component (PC1) calculated over the mean values of the Mosimann shape variables (ratio). The variables group the 40 South American human populations studied by diet CARB = carbohydrate-rich diets (diets 1 and 2); and diet PROT = terrestrial-protein-based diets (diets 3 and 4).

Table 4 Results of the Δ divergence rate test (Lynch, 1990) on size and shape variation for groups from different geographical regions and spatial scales.

Population	Consistent with drift			
	GM	PC1	PC2	PC3
All	No	No	No	No
North	Yes	Yes	Yes	Yes
South	No	No	Yes	Yes
Ecuador and Peru	Yes	Yes	No	Yes
Venezuela and Colombia	Yes	Yes	Yes	Yes
Southern Brazil and Chaco	No	No	Yes	Yes
North-west Argentina, Bolivia and Chile	Yes	Yes	Yes	Yes
Patagonia	No	No	Yes	No

The table indicates whether the result is consistent with a genetic drift model (Yes/No). Size is described using the geometric mean (GM) for all cranial measurements. Shape is described using the first, second and third principal component score (PC1, PC2, PC3). Results were obtained for all 40 South American human populations studied.

(the maximum value expected by genetic drift for mammalian skeletal traits; Lynch, 1990) for the conservative 928 generations since divergence. Similar results were achieved for the morphological divergence among populations from our South region (Table 4). The results for overall cranial size (GM) and shape (PC1) showed high divergence, with an estimated number of generations since divergence greater than 1500–1700 (c. 25,000 years) for the null hypothesis of genetic drift to be accepted (Fig. 6). However, the Δ divergence rate test suggests that non-random processes are less important in relation to the shape variation described by PC2 and PC3 (Table 4), particularly at the regional (North and South) scale. In addition, size and allometric shape (PC1) divergence among populations at a smaller scale – where ecological variation is low (Venezuela and Colombia; Ecuador and Peru; Bolivia; and northern Chile and north-western Argentina) – could be explained by random processes alone (Figs 1 & 2).

DISCUSSION

Our results show that cranial variation in South America is spatially structured and characterized by positive autocorrelation at short distances (i.e. greater similarity among neighbouring populations) coupled with negative values at large distances (Fig. 3). The existence of spatial structure in craniofacial variation among South American populations was also shown in previous studies, which demonstrated that the geographical distance between local populations was correlated with the morphometric distance between them (Pucciarelli *et al.*, 2006; Perez *et al.*, 2007b; Varela *et al.*, 2008). In addition, several molecular studies have shown the existence of spatial structuring in the evolutionary relationships among South American populations (Merriwether *et al.*, 1995; Moraga *et al.*, 2000; Fuselli *et al.*, 2003).

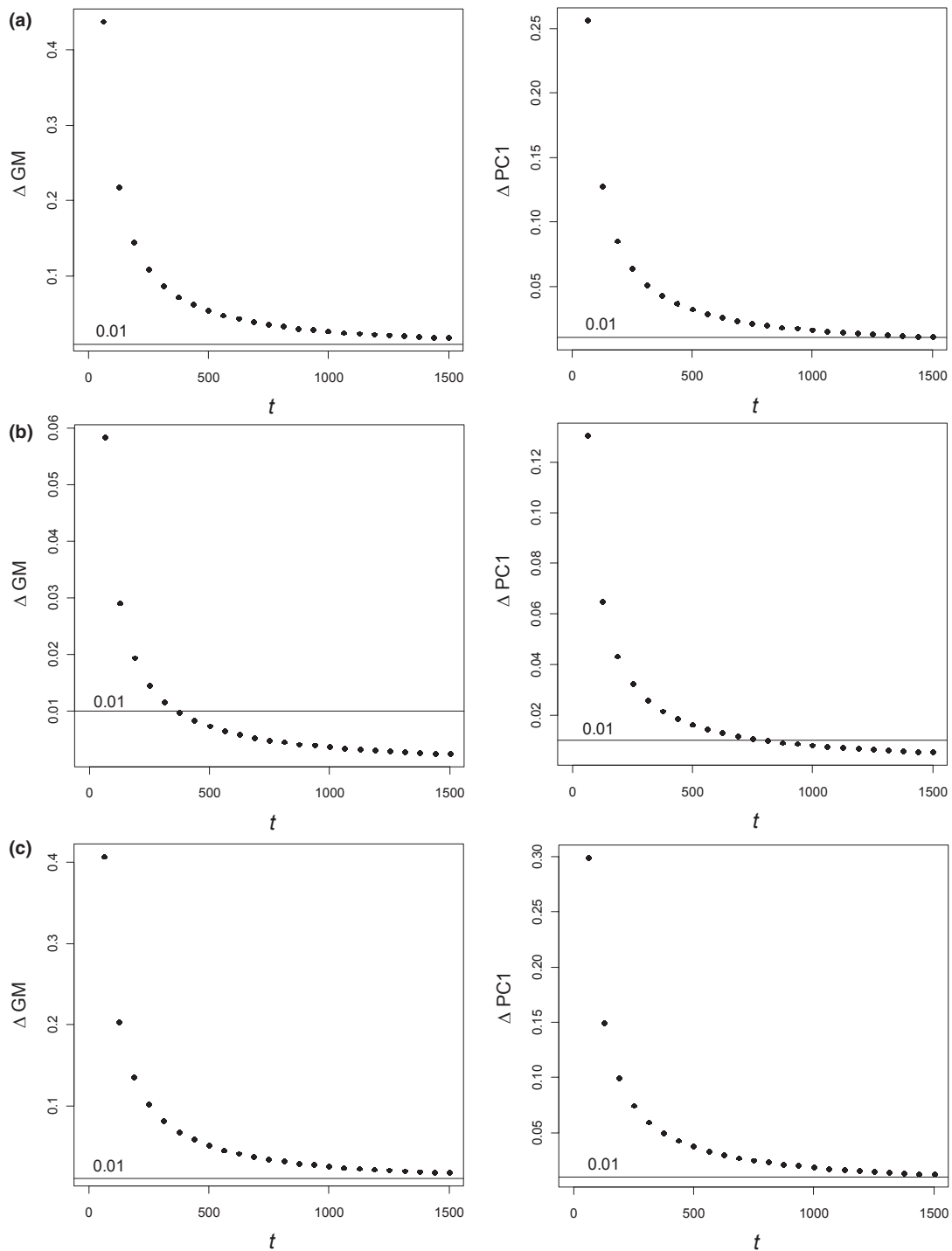


Figure 6 Results of Lynch's (1990) Δ divergence rate for size (geometric mean, GM) and shape (principal component 1, PC1) variation among (a) all samples (40 South American human populations); (b) North samples; (c) South samples (see Table 1), using an estimated range of generations since divergence (t).

This spatial structure in cranial size and shape could be related to evolutionary processes such as directional gene flow or serial founder effects (Moraga *et al.*, 2000; Fuselli *et al.*, 2003). In particular, migration from one side results in a well defined cline, leading to high genetic affinity between groups in close geographical proximity as well as greater genetic

differentiation of more distant groups (Sokal *et al.*, 1989). This pattern of variation could be related to the process of human dispersion throughout South America. Alternatively, the clinal pattern of similarity in craniofacial variation could also be driven by variation in local and general environmental variables (Sokal *et al.*, 1989) such as temperature or diet.

The spatial pattern found here shows that close populations are more similar to each other than expected by chance alone (Legendre, 1993; Ives & Zhu, 2006; Perez *et al.*, 2010). Accordingly, spatial autocorrelation between these populations must be taken into account to avoid spurious interpretations of relationships between morphometric variation and environmental variables. To solve this problem, we used a hierarchical multigroup comparison approach in which the Δ value is calculated at multiple spatial scales (Lynch, 1990; Ackermann & Cheverud, 2002; Steppan *et al.*, 2002) and a spatial regression technique (GLS) that incorporates the spatial autocorrelation structure into the residuals of the regression models (Ives & Zhu, 2006; Bini *et al.*, 2009; Perez *et al.*, 2010).

The results of the Δ divergence rate test strongly suggest that random processes are not the main factor driving the cranial size and allometric shape (PC1 score) divergence among South American populations. Specifically, the test shows that the magnitude of size and shape variation among populations at the sub-continental scale, within the generations considered, is too large to be generated by genetic drift alone (Table 4). This is relevant because we used a conservative maximum number of generations since divergence, assuming that the populations have been isolated from each other since the initial peopling of the continent. However, neighbour populations may be related by gene flow or have lower divergence time (as shown by the autocorrelation analyses), which could decrease the power of the tests (Lynch, 1990; Hendry & Kinnison, 1999; Perez & Monteiro, 2009).

The large magnitude of divergence among South American populations in relation to genetic drift expectations, particularly in the southernmost region, has been supported previously by the results of Lande's rate test and by the comparisons of craniometric and molecular F_{ST} values available in the literature (Cavalli-Sforza *et al.*, 1994; Sardi *et al.*, 2005; Perez & Monteiro, 2009). In particular, the F_{ST} values estimated for craniofacial variation in southern South America were larger than those for other datasets (protein and blood group data; Sardi *et al.*, 2005; Perez & Monteiro, 2009). Considering the magnitude of the divergence – mainly in southern South America, where at least 25,000 years of divergence would be necessary for the null hypothesis of genetic drift to be accepted – these results suggest that ecological and non-random factors are paramount to explaining cranial size and shape diversification in this region.

The spatial regression analyses show that the environmental variables explain from 30% to 68% of the overall size and allometric shape variation among populations (Tables 2 & 3). However, the spatial regression analyses show that the PC2 and PC3 variables are not associated with the environmental variables. In particular, diet has a significant effect on the patterns of size and allometric shape variation at the sub-continental scale (Table 3), explaining 68% of size variation and 42% of allometric shape variation among populations. Diet shows the highest slope value, and the largest crania were found among hunter-gatherer groups (Figs 4a & 5). While size variables show the strongest and most significant association

with diet, diet has a lesser effect on the patterns of overall facial and neurocranial shape variation among populations (Table 2; see Appendix S2). These results are relevant to evaluate the independent contribution of these ecological factors, given that Spearman's correlation (r_s) between diet and temperature is low ($r = 0.26$), showing lack of correlation between these variables. Strong association between these variables is a general problem in studies about the relationships between climate and body size (Meiri & Dayan, 2003), and a particular issue in world-scale studies of human populations. The correlation between morphology and temperature might not indicate direct causation if craniofacial size variation is related to diet and there is a correlation between the latter variable and temperature.

Starting at 2000–3000 years ago, South American populations developed diverse subsistence strategies in different geographical areas (Harlan, 1971; Pearsall, 1992, 2008; Erickson, 2008; Fig. 1). These ranged from horticulture and agriculture, based on several domesticated plants (maize, potato, manioc, beans) (Pearsall, 1992; Chonchol, 1996; Piperno & Pearsall, 1998; Barghini, 2004; Mazoyer & Roudart, 2006), to quite specialized pastoralist economies (based mainly on camelids), through hunter-gatherer strategies focused on marine (shellfish, fish, mammals) or terrestrial (mammals, birds, fruits) resources. The transition to food production has been linked to consumption of softer diets, due to changes in food sources and/or processing techniques that resulted in reduced mechanical loadings and consequent size reduction of masticatory structures (Carlson & Van Gerven, 1977; González-José *et al.*, 2005). However, this hypothesis is difficult to test at the South American spatial scale because all Late Holocene human groups possessed food preparation techniques – mainly pottery, basketwork and grinding stone tools (Silverman & Isbell, 2008; Bernal *et al.*, 2010) – potentially able to generate softer diets to similar degrees.

The main difference among the subsistence strategies in South America is the greater availability of carbohydrates related to horticultural and agricultural practices, when compared with the larger proportion of proteins consumed by hunter-gatherer groups (Harlan, 1971; Pearsall, 1992; Scheinsohn, 2003). Increase in the dietary proportion of carbohydrates among Holocene human populations has been documented to have caused a decrease in body and skull size (Bogin & Rios, 2003; Larsen, 2006; Stynder *et al.*, 2007). Size changes related to increased consumption of carbohydrates have also been widely documented among extant human populations (Frisancho, 2009) and rodents (Pucciarelli, 1980; Pucciarelli & Oyhenart, 1987; Cesani *et al.*, 2006). These studies have shown that protein and protein-calorie malnutrition generate significant differences in size (smaller bodies with lower protein consumption) and associated allometric shape changes (Pucciarelli, 1980; Pucciarelli & Oyhenart, 1987; Frisancho, 2009). Specifically, these works have shown that animals that undergo malnutrition present simultaneous reduction of facial dimensions and increase of relative cranial breadth. This pattern of variation matches the shape change

observed in the study area, and is similar to changes that previous studies have attributed to temperature (Roseman, 2004; Harvati & Weaver, 2006; Hubbe *et al.*, 2009). Our results also show that size variation is more related to environmental variation compared with shape variation, as expected, given that the former is more sensitive to environmental variables such as diet (Pucciarelli, 1980; Pucciarelli & Oyhenart, 1987; Frisancho, 2009). The proximate causes of the decrease in size, and of allometric shape changes, can be related to changes in hormonal pathways due to the availability of nutrients required for growth (Duan, 1998; Nijhout, 2003). Somatic growth is controlled primarily by the growth hormone (GH) and the insulin-like growth factor-I (IGF-I) axis. Specifically, a diet with a low proportion of proteins and/or carbohydrates generates resistance to GH action at the tissue level, due to a decline in the production of IGF-I, therefore affecting growth (Duan, 1998; Nijhout, 2003).

CONCLUSIONS

In this work we used two complementary approaches: the spatial regression technique that incorporates the spatial autocorrelation of morphological variables directly into the modelling process (Dormann *et al.*, 2007; Diniz-Filho *et al.*, 2009b; Perez *et al.*, 2010), and quantitative genetic models (Lynch, 1990; Perez & Monteiro, 2009). This combined approach allowed us to show that, although local factors such as neutral processes or local environmental conditions may be important to explain spatial interpopulation differentiation in cranial morphology at a local scale (among neighbour populations), variation in cranial size and allometric shape is significantly correlated with diet at the South American scale. Therefore we can suggest that diet played an important role in driving morphological diversification among these populations. We also show that the change in diet that took place in South America around 3000 years ago generated a large morphological divergence over a relatively short time-scale (a few thousand years). These results highlight the importance of considering that the morphology of modern human populations can evolve more quickly – through developmental response during ontogeny (ecophenotypic response) – when confronted with rapid environmental change, and call into question the role of neutral processes as the most important factors responsible for human morphological diversification at all geographical scales. Finally, we note that further studies like this one will contribute to better understanding of the role of ecological factors in driving morphological variation among populations, which is a fundamental step to increasing knowledge concerning the multiple factors responsible for the morphological diversification of *Homo sapiens*.

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SUPPORTING INFORMATION

Additional Supporting Information may be found in the online version of this article:

Appendix S1 Craniometric variables used in this study.

Appendix S2 Multiple and multivariate regression analyses of facial and neurocranial skeletons.

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