Effect of Bite Force and Diet Composition on Craniofacial Diversification of Southern South American Human Populations

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ABSTRACT   Ecological factors can be important to shape the patterns of morphological variation among human populations. Particularly, diet plays a fundamental role in craniofacial variation due to both the effect of the nutritional status—mostly dependent on the type and amount of nutrients consumed—on skeletal growth and the localized effects of masticatory forces. We examine these two dimensions of diet and evaluate their influence on morphological diversification of human populations from southern South America during the late Holocene. Cranial morphology was measured as 3D coordinates defining the face, base and vault. Size, form, and shape variables were obtained for 474 adult individuals coming from 12 samples. Diet composition was inferred from carious lesions and δ^{13}C data, whereas bite forces were estimated using traits of main jaw muscles. The spatial structure of the morphological and ecological variables was measured using correlograms. The influence of diet composition and bite force on morphometric variation was estimated by a spatial regression model. Cranial variation and diet composition display a geographical structure, while no geographical pattern was observed in bite forces. Cranial variation in size and form is significantly associated with diet composition, suggesting a strong effect of systemic factors on cranial growth. Conversely, bite forces do not contribute significantly to the pattern of morphological variation among the samples analyzed. Overall, these results show that an association between diet composition and hardness cannot be assumed, and highlight the complex relationship between morphological diversification and diet in human populations. Am J Phys Anthropol 155:114–127, 2014.   © 2014 Wiley Periodicals, Inc.

Ecological factors can shape the patterns of morphological variation among populations either because they act as a selective pressure during the divergence of populations spatially localized in specific environmental contexts or because organisms respond to environmental influences during ontogeny (phenotypic plasticity or eco-phenotypic responses; Schluter, 2000; Roseman, 2004; Collard and Wood, 2007). In particular, morphological change among populations with a recent divergence and occupying heterogeneous environments can result from directional selection and phenotypic plasticity rather than processes such as genetic drift and mutation (Carroll et al., 2007; Perez and Monteiro, 2009).

Accordingly, patterns of variation in modern humans at a worldwide scale show that—although the overall cranial variation is mainly geographically structured and probably related with random processes—some cranial traits are associated with environmental variables such as temperature (Harvati and Weaver, 2006; Hubbe et al., 2009). Several studies at regional scales have also noted the association of craniofacial variation with broad and dichotomous diet categories, with hunter-gatherers having bigger and more robust skulls than farmers (Carlson and van Gerven, 1977; Stynder et al., 2007; Perez and Monteiro, 2009; Paschetta et al., 2010). This has been attributed to differences in masticatory force or loading due to the consumption of items that differ in their mechanical properties, with farmers relying on softer and more processed diets than hunter-

gatherers (e.g., Carlson and van Gerven, 1977; Paschetta et al., 2010). In this context, a reduction in the mechanical loading will result in an underdevelopment of masticatory muscles and the associated cranial structures. Others have stressed the role of diet composition as a factor with systemic effects on the skull (Stynder et al., 2007; Perez and Monteiro, 2009). Because diets poorer in proteins characteristic of farmer groups (Larsen, 2006) tend to decrease overall growth, this might also result in cranial differences due to allometric changes (Perez and Monteiro, 2009). The

influence of these two dimensions of diet on cranial variation is well supported by a large body of experimental evidence on rodents, hyraxes, and nonhuman primates (Beecher et al., 1983; Pucciarelli and Oyhenart, 1987; Dressino and Pucciarelli, 1997; Lieberman et al., 2004). However, both might have similar effects on cranial morphology (i.e., reduction in size and robusticity of malar and maxillar bones with the consumption of artificially softened foods, as well as with diets poor in proteins), and thus, their role on cranial variation in particular human populations is difficult to assess based on morphometric data only.

The lack of comparative studies that explicitly take into account and quantify independently the multiple dimensions in which diet can be decomposed, such as type and amount of nutrients and the physical properties of food items, have limited the comprehension of the processes underlying the observed association between diet and cranial morphology. Southern South America is an excellent region for investigating the process of cranial diversification at regional scales in relation to dietary factors because these populations occupied a wide range of environments, which differ in the amount and type of the available resources. This ecological variation increased during the last 3,000 years as a consequence of the development of food production (i.e., agriculture) and processing technologies (i.e., pottery and grinding tools) leading to changes in diet composition and masticatory loads (Harlan, 1971; Pearsall, 1992, 2008). In addition, the exceptionally high levels of cranial variation found among South American populations, comparable to geographically larger regions worldwide (Sardi et al., 2005; Bernal et al., 2006), might have arisen locally since the molecular evidence suggests that they descend from a single ancestral population, which colonized the region 13,000 years BP (García-Bour et al., 2004; de Saint Pierre et al., 2012). Previous studies concerned with the causes of these high levels of cranial variation among native populations from southern South America disagree on the influence of carbohydrate/protein intake and masticatory loads, although these two dimensions of the diet have not been thoroughly evaluated (Gonzalez-José et al., 2005; Perez and Monteiro, 2009; Perez et al., 2011).

The main objective of this study is to investigate the contribution of diet composition and bite forces on the craniofacial diversification of late Holocene human populations from the southern region of South America. Particularly, we expect that if the pattern of craniofacial variation is related to differences in masticatory loading, the morphometric variation will be correlated with bite force estimations. Alternatively, if craniofacial variation is related to diversity in diet composition, the pattern of morphometric variation among populations should be correlated with differences in the proportion of carbohydrates and proteins consumed. Localized effects in the skull are expected if the masticatory loading rather than diet composition was the main factor underlying the cranial diversification in the region under study (Pucciarelli and Oyhenart, 1987; Lieberman et al., 2004). This was evaluated by analyzing the whole skull and three previously hypothesized modules that differ functionally and developmentally: face, vault, and base (Cheverud, 1995; Lieberman et al., 2000; Sperber, 2001; Hallgrímsson et al., 2007; Perez and Monteiro, 2009). We quantified cranial morphometric variation (form, size, and shape) using coordinates of reference points (i.e., landmarks and semilandmarks) and geometric morphometric techniques (Adams and Otarola-Castillo, 2013). We measured diet composition (i.e., relative percentage of carbohydrate and protein) using dental caries and carbon isotopes; and infer masticatory loading using bite force estimations based on cross-sectional areas of the main jaw adductor muscles and their respective in-levers about the temporomandibular joint (TMJ; Kiltie, 1984; Thomason, 1991; Christiansen and Wroe, 2007). Because cranial variation among neighbor populations in this geographic region displays spatial structure due to the effect of neutral processes (Perez et al., 2010, 2011; Bernal et al., 2010; de Saint Pierre et al., 2012), the association of cranial variation with diet composition and bite force was tested by using spatial comparative methods (Legendre and Legendre, 1998; Diniz-Filho et al., 2007).

MATERIAL AND METHODS

Samples

For this study, we analyzed skulls with no evidence of artificial cranial modifications from 12 samples (N individuals = 474) of male and female adults. The 12 samples come from three geographic regions (Fig. 1): Northwestern Argentina (NW, $N = 183$), Southeast Pampa/Northeastern Patagonia (SP/NP, $N = 127$), and Central Western Argentina (CW, $N = 164$). Samples are deposited in Museo de La Plata, Museo Etnográfico "J.B. Ambrosetti" of Buenos Aires, Museo de Ciencias Naturales y Antropológicas "Juan Cornelio Moyano" in Mendoza, Museo Regional de Malargüe, Museo de Historia Natural in San Rafael and Instituto de Investigaciones Arqueológicas y Museo "Prof. Mariano Gambier." Table 1 shows more details about the samples studied by geographical region.

Sex and age determinations were done using osteological criteria (Buikstra and Ubelaker, 1994). Given that the samples belong to museum collections, the majority of individuals were represented by skulls only, and therefore the determinations were restricted to cranial traits. Age estimation was based on inspection of spheno-basilar suture closure and eruption of M^3 (Buikstra and Ubelaker, 1994). Individuals were assigned to one of two categories, subadult and adult, considering adults as those individuals that had M_3 erupted and the spheno-basilar suture already closed. Individuals with malformations, pathologies or severely reabsorbed alveolar ridge due to ante-mortem tooth loss, among others, were excluded from this study. Sex estimation was done through geometric morphometric methods, which proved to be more useful to describe subtle differences or intermediate morphologies in sexually dimorphic structures (Gonzalez et al., 2011). Coordinates of landmarks and semilandmarks were obtained to describe the glabella, malar, frontal, mastoid, and zygomatic processes. These structures were chosen because they exhibit high levels of preservation in archaeological contexts and the fact that previous studies suggest that these structures are sexually dimorphic in our region of study (Gonzalez et al., 2011). The procedure of landmarks digitization and preliminary data management will be described later. The software used to analyze sexually dimorphic structures was Morphologika 2.5 (O'Higgins and Jones, 2009).

Fig. 1. Southern South America Map depicting the central geographic location of the 12 samples analyzed (Pu: Puna, QY: Quebrada de Yacoraite, QH: Quebrada de Humahuaca, CalV: Calchaquí Valley, AnV: Andalgalá Valley, SJ: South San Juan, NM: North Mendoza, SH: South Mendoza, Pa: Pampa, SB: San Blas, NV: Negro Valley, and ChV: Chubut Valley). More detailed information of the samples is shown in Table 1.

Morphometric analyses

A total of 84 three-dimensional (3D) anatomical points (42 landmarks and 42 semilandmarks) located over the whole skull were defined for this study (Fig. 2). Landmarks were selected according to Howells (1973) and Buikstra and Ubelaker (1994). Semilandmarks were placed mainly along craniofacial sutures and contours. Data were collected employing a MicroScribe G2X digitizer. The 3D points for each specimen were aligned using an extension of the Generalized Procrustes Superimposition (GPS) method (Bookstein, 1997; Adams et al., 2004). This method aligns the configurations of landmarks and semilandmarks using a least square criteria, and minimize the differences tangent to the contours in the case of semilandmarks (Bookstein, 1997; Perez et al., 2006). Specifically, semilandmarks were slid along the curves by minimizing the Procrustes distances between the points, until they coincided with the positions of the corresponding points along the contour in the reference configuration. For the purposes of our study, this procedure is done because the curves or contours should be homologous among individuals, although not necessarily their individual points need to be (Mitteroecker and Gunz, 2009).

We then studied three geometric morphometric properties: shape, size, and form as defined by Rohlf and Slice (1990) and Mitteroecker et al. (2004). Shape was analyzed using Procrustes coordinates, that is, the Cartesian coordinates after GPS alignment. Size was measured as the logarithm of centroid size (CS), which was calculated as the square root of the sum of squared distances of all points from its center of gravity. Cranial form was described as the Procrustes coordinates plus logCS (Mitteroecker et al., 2004; Mitteroecker and Gunz, 2009). A principal component analysis (PCA) was performed over all the Procrustes coordinates (shape variables), and over Procrustes coordinates plus logCS (form variables), to obtain low-dimensional axes of shape and form variation, respectively. All analyses were performed on the mean value of each sample, using the average of individual CS and the consensus configuration based on Procrustes coordinates.

The morphometric analyses were done for the entire skull and for three modules—face, vault and base—that

TABLE 1. *The 12 samples from which the South American human populations were studied, together with their abbreviations, sample sizes (N), mean* ^{14}*C dates,* δ^{13}*C, CI, and geographic region*

Geographic región	Sample	Abrev.	N	mean^{14}C	δ^{13}C	CI
Northwestern Argentina (NW)	Quebrada de Yacoraite	QY	27	700 ± 60	11.2	15
	Andalgalá Valley	AnV	22	400 ± 50	12.4	13.1
	Puna	Pu	45	700 ± 60	15.8	12.16
	Calchaquí Valley	CalV	46	821 ± 40	11.8	9.34
	Quebrada de Humahuaca	QH	43	910 ± 50	11.2	12.5
Central Western Argentina (CW)	South San Juan	SJ	59	559 ± 40	13.3	10
	North Mendoza	NM	46	1080 ± 45	14.8	2.75
	South Mendoza	SM	59	1766 ± 50	16.6	3.87
Southeast Pampa/Northeastern Patagonia (SP/NP)	San Blas	SB	32	1028 ± 45	16.85	1.1
	Pampa	Pa	35	1300 ± 40	16.5	9.8
	Negro Valley	NV	24	523 ± 45	17.98	0.9
	Chubut Valley	ChV	36	1326 ± 65	16.6	3.5

Radiocarbon age (AMS) and δ^{13}C determination for South San Juan was held at the Arizona Radiocarbon AMS Facility (University of Arizona) from collagen of a rib bone fragment.
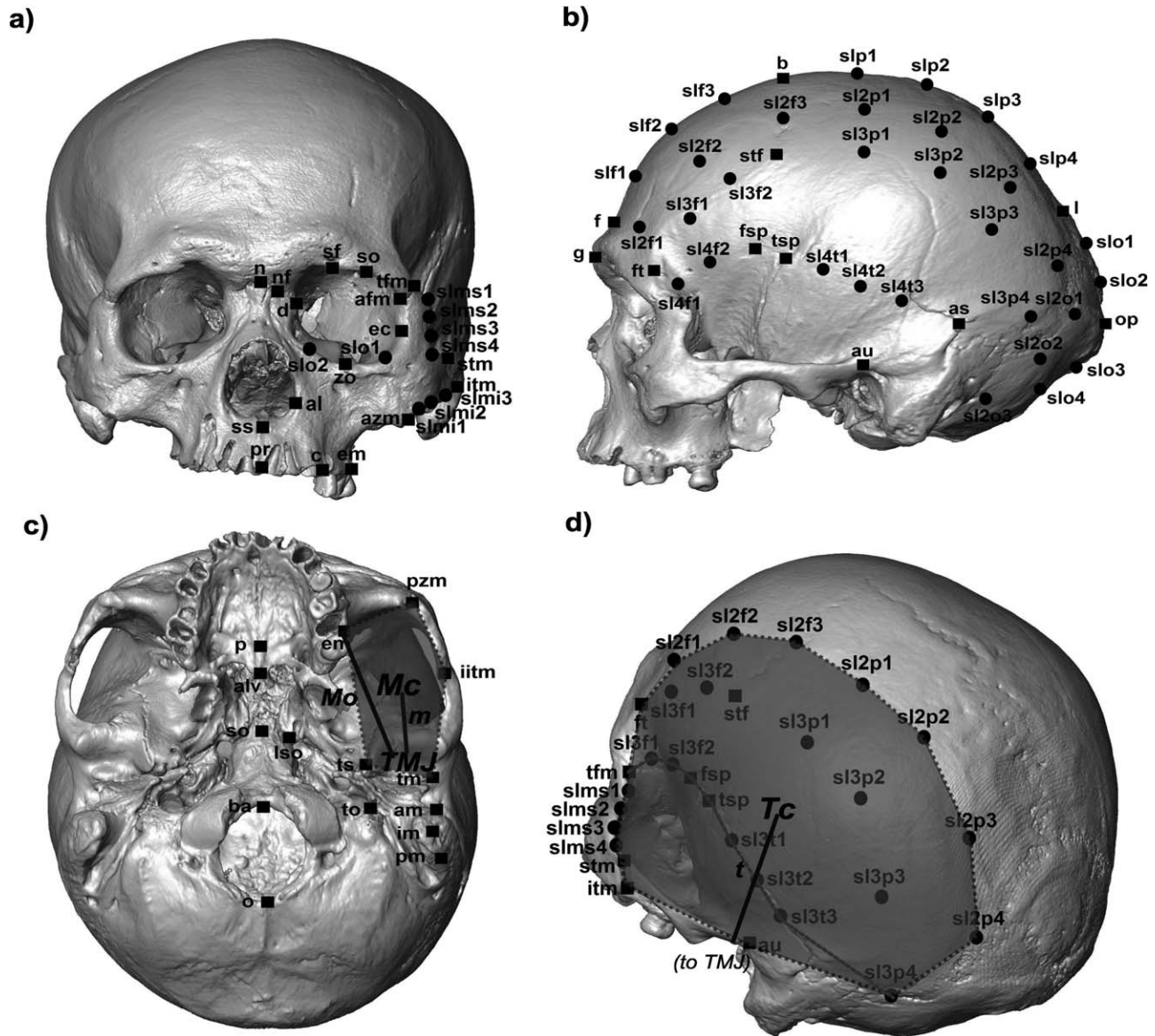
Fig. 2. Landmarks (L), Semilandmarks (Sl) and muscles registered for the present study by cranial region. (**a**) Face landmarks: n: nasion, nf: nasofrontal, ss: subespinal, al: alar, d: dacrion, sf: supraorbital foramen, so: superior orbital, afm: anterior frontomalar, ec: ectoconchion, zo: zigoorbital, pr: prosthion, c: canine, em: ectomolar, p: palatine, alv: alveolon, tfm: temporal frontomalar, stm: superior temporomalar, itm: inferior temporomalar, iitm: intern inferior temporomalar, azm: anterior zygomaxillar, and pzm: posterior zygomaxillar; (**b**) Vault landmarks: g: glabella, f: frontex, b: bregma, l: lambda, op: opistocranion, ft: frontotemporal, stf: stefanion, fsp: frontosphenoparietal, tsp: temporosphenoparietal, and as: asterion, (**c**) Base landmarks: o: opisthion, ba: basion, so: sphenooccipital, lso: lateral sphenooccipital, to: temporooccipital, ts: temporosphenoides, tm: temporomandibular, au: auricular, am: anterior mastoides, im: inferior mastoides, pm: posterior mastoides. Masseter muscle (gray area) demarcated by landmarks, masseter inlever arm (m), outlever arm (Mo), and centroid of masseter (Mc); (**d**) Temporal muscle (gray area) demarcated by landmarks and semilandmarks, temporal inlever arm (t), and centroid of the temporal muscle (Tc).

represent developmentally and functionally units that vary with relative independence from one another (Cheverud, 1995; Lieberman et al., 2000; Perez and Monteiro, 2009). Particularly, the face is related to food processing and then it is expected to display the closest association with environmental variables as diet (Cheverud, 1995; Sperber, 2001; Gonzalez-Jose et al., 2005), whereas the cranial base would be the structure least related to these factors (Lieberman et al., 2000; Sperber 2001; Harvati and Weaver, 2006; but see Roseman et al., 2010).

A subsample of 361 individuals were used for the analysis of the entire skull, face, vault, and base. Facial analyses were repeated using the total of 474 individuals. This discrepancy in the number of individuals responds to differences in bones preservation. There was an attempt to increase the number of individuals included in the facial analysis due to the importance of this module for the present study. Morphometric analyses were done with MorphoJ (Klingenberg, 2011) and package Geomorph of R software (Adams and Otarola-Castillo, 2013; R Developmental Core Team, 2013).

TABLE 2. Principal Axis results for evaluating the association between DW and CI

Groups	r^2	P	Intercept	Slope	Angle
QY	0.89	0	0.089	0.752	36.955
AnvV	0.6	0.01	3.234	0.659	33.407
Pu	0.5	0	3.287	0.554	29.029
CalV	0.27	0.05	9.453	0.338	18.707
QH	0.8	0	−6.860	1.170	49.482
Pa	0.52	0	−1.685	0.902	42.053
SSJ	0.43	0	−6.498	1.172	49.540
NM	0.65	0	0.546	0.685	34.412
SM	0.68	0	−10.760	1.121	48.290
SB	0.72	0	−2.030	0.860	40.702
NV	0.55	0	−19.640	1.485	56.054
ChV	0.44	0.01	3.681	0.617	31.680

Diet variables

Diet composition. Diet composition was measured using two independent variables widely used in archaeological and bioarchaeological studies, caries and carbon isotope data. While caries inform about the amount of carbohydrates consumed, the isotope data used (collagen $\delta^{13}C$) informs about the protein fraction of the diet. For this reason, we considered these two variables separately.

Stable isotopes of the inorganic and organic fractions of bone provide a good record of dietary intake over at least the last 5–7 years of an individual's life, and thus can be used to infer the average diet in human prehistoric populations (Ambrose and Krigbaum, 2003; Tykot, 2006). Particularly, carbon isotope ($\delta^{13}C$) values of collagen reflect the origin of the protein component of the diet and allow to assess the contribution of C_3 or C_4 plant foods. In this work, stable isotopic values were obtained from published data as well as data obtained in this study (Table 1; Bernal et al., 2008; Berón et al., 2009; Gil et al., 2009, 2011; Beguelin, 2011; Gordon, 2011; Killian Galván and Samec, 2013). Only $\delta^{13}C$ collagen values were employed because there is a wider collection of this data available.

Dental caries are the result of a demineralization process on tooth tissues due to the production of organic acids from bacterial fermentation (e.g., *Streptococcus mutans*) of carbohydrates (Hillson, 2001). Consequently, they provide valuable information about the proportion of carbohydrates, sugar, and sticky nutrients in the diet of prehistoric populations (Larsen et al., 1991; Lukacs, 1992). In this work, the presence of carious lesions was recorded by us (V.B., P.N., and L.M) following Buikstra and Ubelaker (1994). Finally, the Carious Index (CI) was calculated as a function between the number of carious teeth and the total number of teeth available (Hillson, 2001).

Dental wear (DW). Given that the prevalence of caries in a sample could be affected by the degree of DW, the latter was recorded to get an indirect control of the quantification of the carious lesions. DW is a continuous long-lasting process that results from the mutual contact of opposite crowns as well as from their contact with food or abrasive material incorporated into food (Smith, 1984), that might remove both fissures of erupting molars before they became carious and the carious tissue as soon as a lesion appears (Hillson, 2000). Considering

the natural eruption sequence of permanent molars, in which there is an approximate 6-year interval between the eruptions of the first, second and third molars in all human populations, it is possible to derive age-independent rates of wear with acceptable confidence (Scott and Turner, 1988). According to this, rates of wear were estimated based on the degree of wear following the ordinal scale proposed by Scott (1979) for M^1 and M^2. Molars showing no exposed dentine or total loss of enamel were excluded from analysis. A principal axis analysis was applied to estimate wear rates between M^1 and M^2. The slope obtained was then used as an indicator of the relationship between adjacent molars, and therefore, wear rate (Scott and Turner, 1988).

We also discriminate DW related to masticatory function from the wear generated by use teeth as tools (i.e., parafunctional use of teeth). We recorded macroscopically the degree, direction and form of individual tooth wear as proposed by Smith (1984) for incisors, canines and premolars, and Scott (1979) for molars. Since the teeth function as a unit, harmonic DW would be attributable to the masticatory function, while atypical tooth wear could indicate the use of teeth as a tool (Larsen, 1997). Therefore, the presence of teeth that are outliers with respect to adjacent ones were considered as probably employed in paramasticatory functions (Molnar, 1971) and the individuals were excluded from the following analyses.

The relationship between DW and CI was explored using correlation analyses. These results show two axes of independent variation given by CI and DW (Table 2), which means that carious lesions are not related to DW ($r = -0.28$). CI is associated to latitude, with the highest values occurring in the NW groups and Pampa, intermediate values in CW samples and the lowest values in the SP/NP group. Conversely, DW does not follow a geographic pattern, presenting the highest values in South San Juan and South Mendoza samples, and the lowest values in Chubut Valley, North Mendoza, and Quebrada Yacoraite.

Bite force (BF). Bite force was estimated using a combination of two models based on cranial anatomy and lever mechanics, proposed by Kiltie (1984) and Thomason (1991), with some modifications to adapt such models to human head anatomy, as well as to 3D geometric morphometrics. By this technique we inferred bite force using (a) an estimation of the size of the major jaw adductors, the temporalis and the masseter-medial pterygoideus muscles complexes, (b) approximate measurements of the muscle moment arms about the TMJ, and (c) moment arms of upper second molar (M^2) about TMJ. The major assumptions made when using these models are that the true physiological cross-section of muscles, which have a complex 3D architecture, can be simplified and that contralateral muscles are assumed to contract equally forcefully (Ellis et al., 2008). We also assume that for humans the upward force of the adductor muscles is balanced by downward forces at three places: the two condyles and the bite point, which for modern human is located between M^2 and M^3. These three points constitute the triangle of support for mastication in *Homo sapiens* (Lieberman, 2011). However, it is important to remark that different versions of this method could generate differences in the bite force estimations (Ellis et al., 2008).

To estimate the muscles size (a), we used a combination of Kiltie (1984) and Thomason (1991) methods, as 3D landmarks allow us to estimate a global measurement of temporalis (Tcs) and masseter-medial pterygoideus (Mcs) muscles size using CS values (see Fig. 2). The moment arm of the temporalis and masseter muscles (b) was estimated following Thomason (1991). According to this, the direction of each force vector was assumed to act perpendicularly to the plane of the area, through its centroid. The in-lever arms of each vector for temporalis (t) and masster (m), were defined as the distance between the centroid of temporalis (Tc) and masseter (Mc) muscles and the TMJ. The centroid of each muscle area was calculated considering the anatomical points displayed in Figure 2 (Mc, Tc). Finally, the out-lever arm to the bite location, was defined as the distance from the TMJ to the junction of M^2 on the maxilla (Mo; Thomason 1991). A factor of 2 was also added on the assumption that both sides were contracting maximally (Ellis et al. 2008).

In summary, the bite force (BF) was calculated using the masseter and temporalis CS (Mcs, Tcs), the in-lever arms from the centroid of each muscle to the TMJ (m, t) and the out-lever arm from the TMJ to the M^2 (Mo), all the above doubled to include both sides of the head:

$$BF = 2[(M_{cs} * m) + (T_{cs} * t)]/Mo$$

The anterior dentition, which was included in Kiltie (1984) and Thomason (1991) calculations, was used here with comparative proposes only because in human beings, its principal function is to cut and tear and not to masticate as the molars do. Therefore, we re-calculated BF with an out-lever arm from the TMJ to the canine (C).

Finally, it was evaluated if there is a linear relation between bite force and cranial size, because these variables are usually related (Kiltie, 1984; Christiansen and Wroe, 2007; Ellis et al., 2009; Herrel et al., 2014). The results of the linear regression between the bite force and logCS for our dataset show that there is no association between there variables in this sample ($R^2 = 0.04$; $P = 0.59$). Therefore, all subsequent analyses were performed with BF values without size standardization.

Spatial data and analysis

Statistical analyses were performed in order to describe the spatial structure of morphometric and ecological variables (Diniz-Filho et al., 2007; Dormann et al., 2007; Perez et al., 2010). Geographic origin of the samples was obtained from already published data and then the geographical coordinates of each local sample were transformed to a geodesic system (decimal degrees of latitude and longitude).

Firstly, Moran's I Correlograms were calculated for the ecological variables ($\delta^{13}C$, CI, and BF) and the morphometric data (CS, shape PC, and form PC) for the entire skull and each of the three modules (face, vault, and base). A correlogram is a graph in which autocorrelation Moran's I values (i.e., a measurement of data similarity based on both data geographic locations and data values simultaneously), calculated at different distance classes, are plotted on the ordinate against distance classes among sampling localities on the abscissa (Sokal and Oden, 1978; Legendre and Fortin, 1989). Moran's I formula behaves mainly like Pearson's correlation coeffi-

cient, with value near +1.0 indicating geographic cluster of data while a value near −1.0 indicating geographic dispersion of data. The shape of the correlogram could be related to the type of spatial structure and evolutionary processes underlying the studied variables (Sokal, 1979). Sokal and Oden (1978) and Legendre and Fortin (1989) gave a series of typical correlogram patterns and the corresponding evolutionary and ecological interpretation. By example, correlograms display a characteristic asymptotically decreasing shape when the pattern of morphometric variation is related to the joint effect of drift and geographically limited gene flow, that is, isolation by distance model (Barbujani, 2000). When other factors are involved, correlograms can show a random pattern, a cline, or a depression. Particularly, clines affecting the entire study area, or only a part of it, can be related to deterministic factors such as natural selection and phenotypic plasticity.

As the main objective of the present work is to evaluate whether bite force and/or diet composition are related to cranial morphology, we performed regression analyses including $\delta^{13}C$, CI, and BF as the independent variables, and CS, shape PC and form PC as the dependent variables. Also, because previous studies pointed out the importance of temperature on cranial variation, this variable was tested in our analyses. Similar to previous studies in the region, we found no association between cranial variation and temperature ($r = 0.00$, $P = 0.9$). One of the assumptions of ordinary least-squares regression model is the independence of observations. This model can be described as y=Xb +ε, where y is the vector that describes morphometric variation, X is the matrix of independent diet variables, b is the vector of regression coefficients, ε is the error term with a covariance matrix **C** among residuals C=σ²I, where σ^2 is the variance of the residuals, and **I** is an identity matrix (Perez et al., 2010). When data are spatially autocorrelated—that is, observations that are closer to each other in space have more similar values— as in our study (see below), autoregressive models instead of the ordinary regression should be used (Legendre and Legendre, 1998). Two autoregressive models were employed here: simultaneous autoregressive model (SAR) and conditional autoregressive model (CAR; Dormann et al., 2007; Perez et al., 2010). In the SAR model, the covariance matrix **C** among residuals is described as $C_{SAR} = \sigma^2\left[(I-\rho W)^T\right]^{-1}[I-\rho W]^{-1}$, where **W** is a weighting matrix based on the inverse of geographic distance and ρ is an autoregressive coefficient for morphometric variable. The CAR model is a modification of the SAR model, where the covariance matrix **C** among residuals is described as $C_{CAR} = \left[(\sigma^2 W_{i+})I\right][I-\rho W]^{-1}$. Therefore, these methods model spatial effects within the error term, which is predefined from a neighborhood matrix, and then parameters are estimated using a generalized least-squares framework (Dormann et al., 2007; Perez et al., 2010). Here, we used simultaneous autoregressive error models with all first order neighbors with equal weighting of all neighbors. The fit of the models was compared by R^2 and AIC values for SAR and CAR.

Prior to regression analysis, Pearson's correlation analyses were performed to test the association among the ecological variables. This was done to avoid problems of multicollinearity. If variables are collinear, or nearly
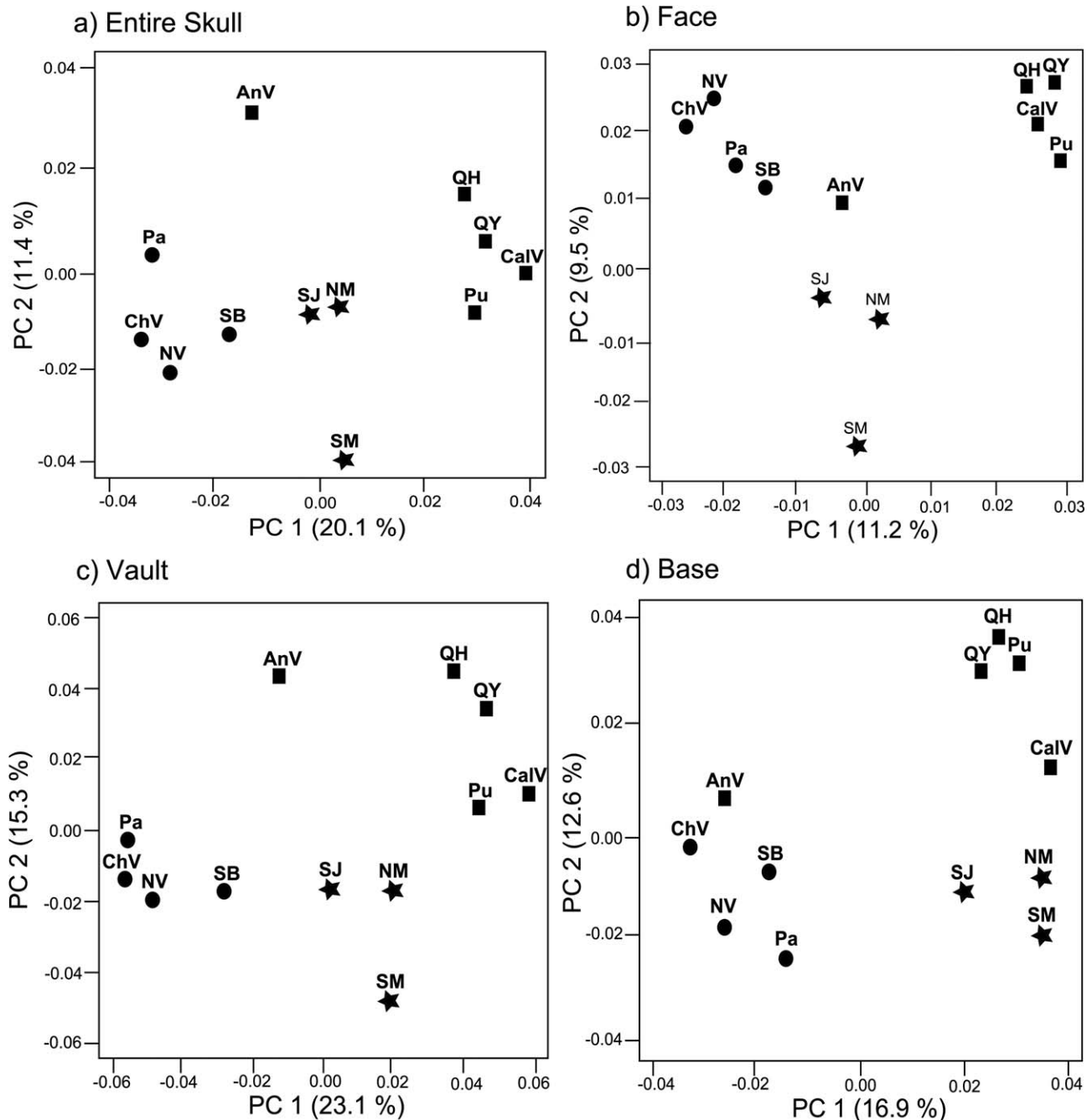
Fig. 3. The results of the distribution of the 12 sample along the first two principal shape PCA axes by cranial region: (**a**) entire skull, (**b**) face, (**c**) vault, and (**d**) base. In brackets is shown the percentage of variation explained by each principal component.

so (i.e., highly correlated), the results obtained are difficult to interpret because the independent effect of each predictor cannot be estimated correctly (Legendre and Legendre, 1998). Pearson correlations indicated that the correlation between BF and CI ($r = -0.224$) and between BF and $\delta^{13}C$ ($r = 0.229$) was low. Conversely, the correlation between $\delta^{13}C$ and CI was high ($r = -0.769$). Because of collinearity between $\delta^{13}C$ and CI, subsequent regression analyses were performed separately for the latter variables. Spatial analyses were done with SAM software (Rangel et al., 2010).

RESULTS

Shape, size, and form

The distribution of samples along shape PC1 follows a geographic pattern, with NW and SP/NP samples on the opposite extremes and CW samples in an intermediate position (Fig. 3). Shape PC2 also separates CW samples from the others. When the entire skull is considered (Fig. 3a), SP/NP samples locate in the positive extreme of shape PC1, NW samples in the negative shape PC1 extreme, while CW samples stay in the middle and differ
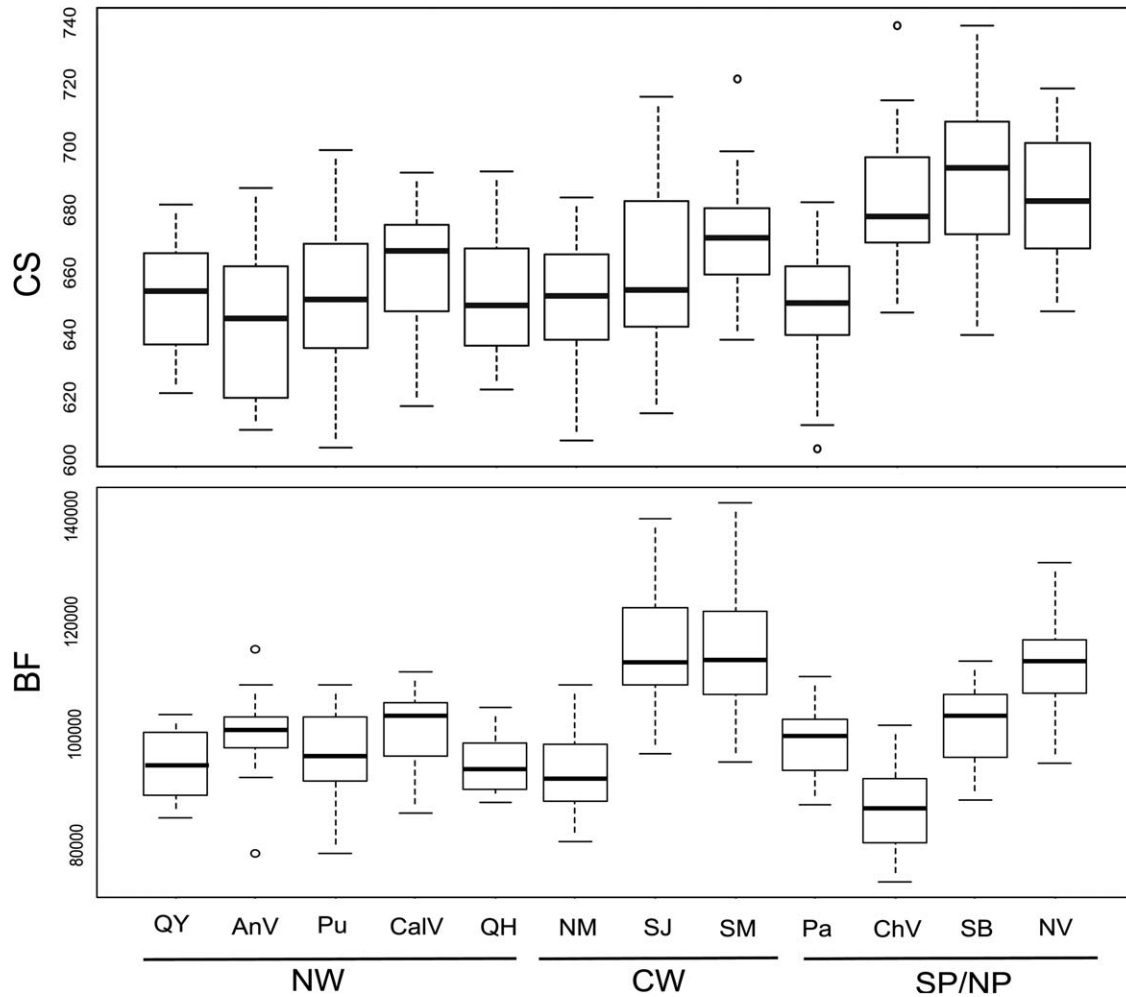
Fig. 4. Boxplots showing variation in CS and BF per sample, which are grouped according to geographical region.

from the other samples along the PC2. The dispersion of the samples in the shape PCA for the facial and vault modules is similar (Fig. 3b,c). The PCA of cranial base (Fig. 3d) displays the highest superposition between NW and CW samples. NW and CW samples locate on the positive extreme of shape PC1, while SP/NP group on the negative one.

The wireframes shown in Supporting Information Figure S1 illustrate the main trend of shape changes along PC 1 axis. Most important changes in the face are related to nasal bones, orbit, and maxilla (Supporting Information Fig. S1a). Skulls located at the negative extreme, from the SP/NP group, display a relative increase along the supero-inferior axis in orbits and nasal bones, and along antero-posterior dimensions in the maxilla. Vault configurations at the positive extreme, from the NW samples, show an increase in the supero-inferior axis and a decrease in the antero-posterior dimensions compared with the samples at the negative extreme (Supporting Information Fig. S1b). The base is the module with fewer changes: in general terms, cranial bases at the negative extreme show a relative increase in antero-posterior dimensions (Supporting Information Fig. S1c).

Variation in cranial size among samples shows a similar geographical pattern as shape (Fig. 4). NW and CW

samples have on average the smallest skulls, while the NP samples display the largest CS values. Form PC also displays a spatial pattern, but the samples are not clustered as strong as in shape PCA (Supporting Information Fig. S2). CW samples are closer together when the entire skull, face and vault were analyzed, and as in shape plots, they tend to locate in an intermediate position between NW and SP/NP samples. In the form PCA for the cranial base, the samples from NW and CW are mixed, different to the shape PCA of cranial base.

The spatial correlograms of size and shape variation are shown in the Figure 5. The Supporting Information Figure 3 depicts the correlogram of form variation. These correlograms show a monotonic decrease in Moran's I coefficient with geographic distance, suggesting a clinal pattern in the spatial distribution of the studied samples. Neighbor samples are more similar than the distant ones, showing a strong morphometric similarity at small geographic scales. This pattern is stronger for the entire skull, vault, and face shape data. Moran's I values are high but not significant, which might be due to the number of samples studied.

Diet variation

CI and $\delta^{13}C$ were plotted with the objective of exploring their relationship to each other. As can be seen from
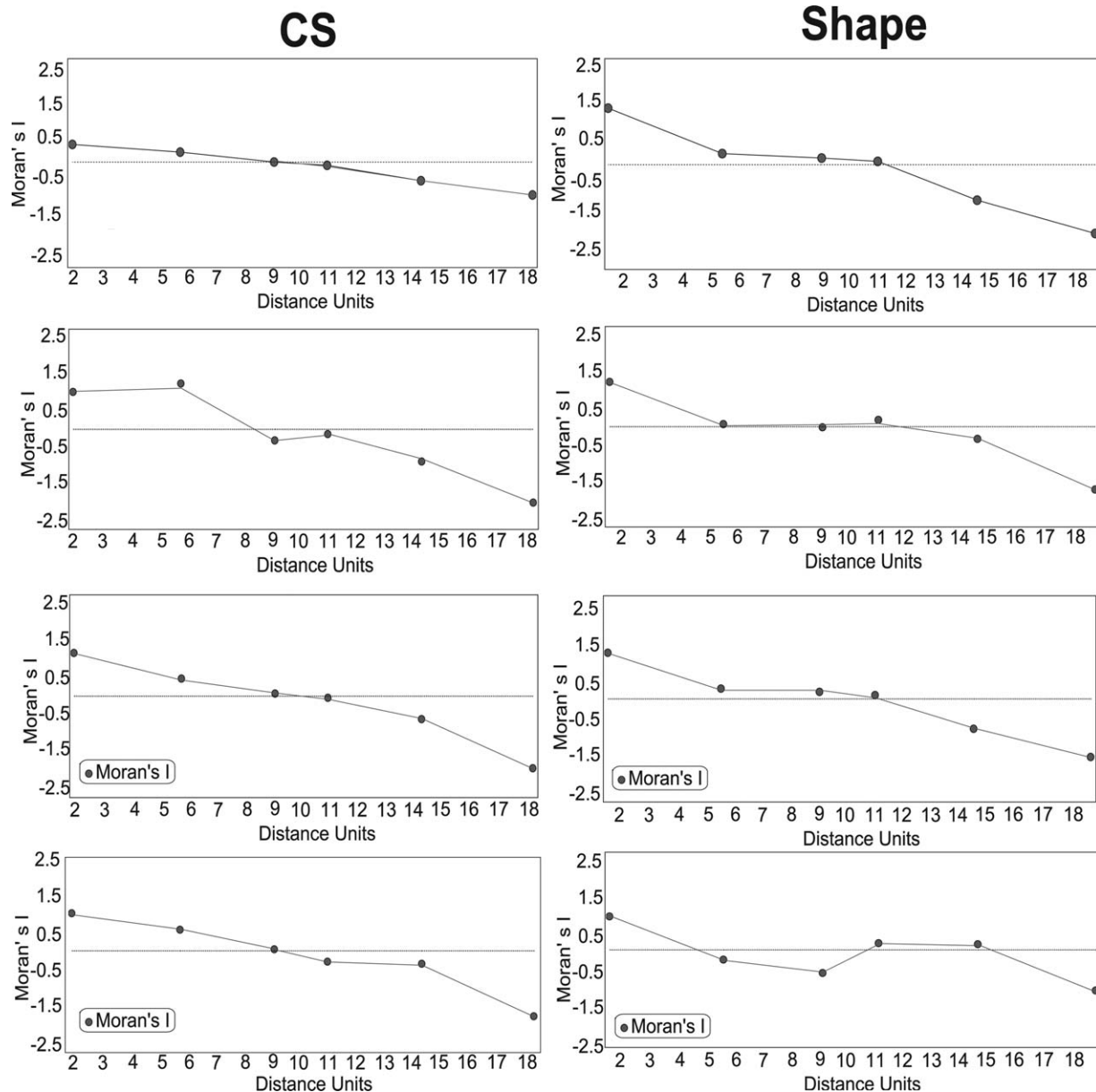
Fig. 5. Morphological Correlograms for CS and Shape by cranial region. Each correlogram mapped a morphological variable to explore its spatial structure and assess spatial autocorrelation. Distance units (geodesic) are shown in the horizontal axes and increase to the right side. Moran's I (autocorrelation coefficient) values are shown on the vertical axes.

Figure 6, in general terms, these variables show a linear relationship. In the extremes of the distribution, North Patagonian samples (NV, SB, and ChV) have the lowest $\delta^{13}C$ and CI values while North West samples (QY, QH, AnV, and CalV) have the highest $\delta^{13}C$ and CI values. CW samples are distributed between these two groups. An exception of this association constitutes Pa and Pu samples, with both having high CI and low $\delta^{13}C$ values. Because $\delta^{13}C$ and CI values probably describe different diet properties, we decided to analyze both variables independently in the subsequent analyses in order to prevent the loss of information.

Bite force boxplot (Fig. 4) shows great variation between extreme values and does not display the same geographical pattern as other variables measured. In general, samples from NW have the lowest values and

CW samples have the highest values. However, SP/NP samples display a range of values that includes the extreme values of NW and CW samples (Fig. 4). Particularly, SM, SJ, and NV have the highest values, while ChV, NM, and QH have the lowest ones. In addition, Pa sample has similar bite force values to NW samples. CW samples have the greatest dispersion. Among NW samples, there are slight differences between the extreme values, with CalV having the highest bite force values and QH the lowest ones. However, a great difference exists between extreme values of SP/NP with NV having the highest values and ChV the lowest ones. Correlograms showed that, contrary to BF, $\delta^{13}C$, and CI display a clinal geographical pattern (Fig. 7), but this pattern is not as strong as for morphometric shape data.

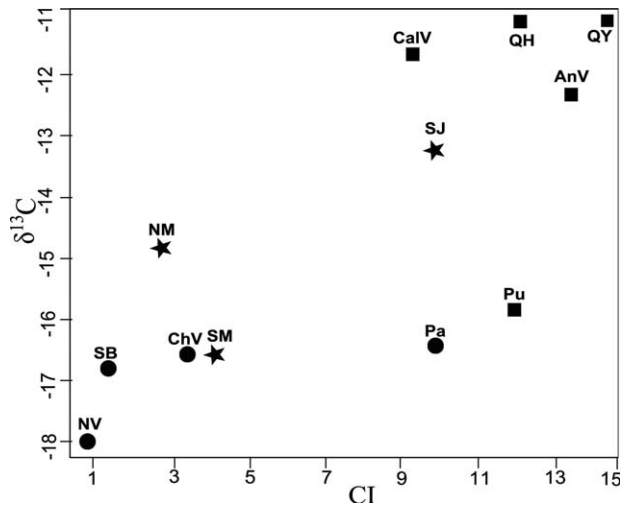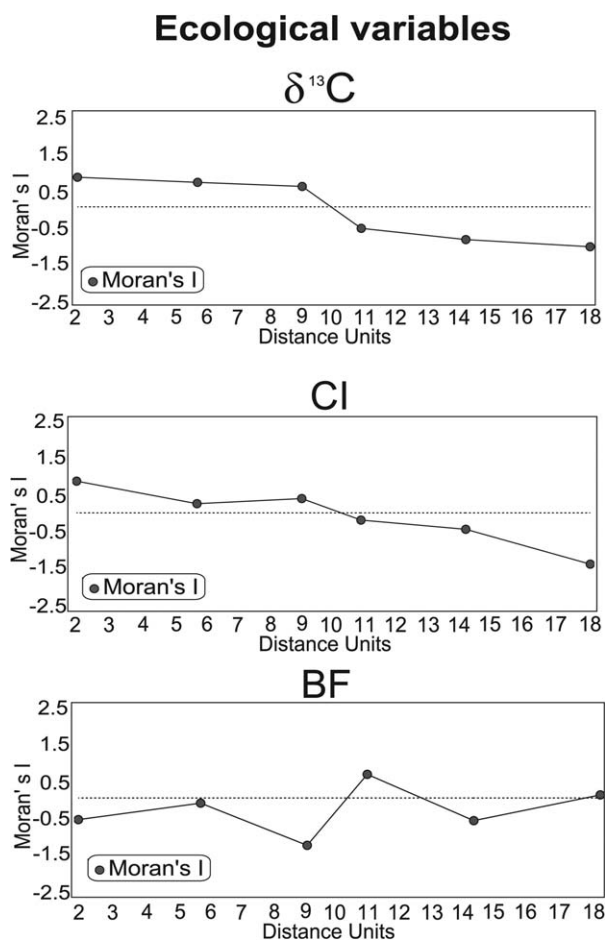**Fig. 6.** Scatterplot for evaluating the association between CI and δ^{13}C.

Fig. 7. Ecological Correlograms for δ^{13}C, CI, and BF. Each correlogram maps an ecological variable to explore its spatial structure and assess spatial autocorrelation. Distance units (geodesic) are shown in the horizontal axes and increase toward the right. Moran's I (autocorrelation coefficient) values are shown on the vertical axes.

Regression models

The regression results (Supporting Information Table S1) are ordered by cranial module and the predictors according to AIC values from the lowest (best fit and simple model) to the highest one. The R^2 values (fit degree) are also displayed. Overall, our results indicate that size (CS) is significantly associated with the ecological variables analyzed here. When the entire skull is considered, diet composition (measured as CI) is the variable that best predicts size variation. Particularly, CI explains 59% of variation in skull size. The same association was found for form variation, displaying 58.9% of fit for entire skull. Similar results were obtained for facial and vault variation, CI being the variable that best predicts their variation in form and size (with values ranging from 30 to 55%). The percentages of variation in the entire skull, facial, and vault shape accounted for the ecological variables were much lower (between 0 and 28%) and nonsignificant. Finally, variation in cranial base size and form was better explained by CI, which accounted for 40 and 30% of total variation, respectively. Conversely, none of the ecological variables accounted for a large amount of variation in cranial base shape. Overall, the samples with highest CI values are more gracile and display the smallest skulls, while the samples with lower CI values are more robust and have the largest skulls. BF alone explains a very low proportion of size, form and shape variation (ca. 0%) when bite forces are estimated based either on canine or second molar (Supporting Information Table S1).

DISCUSSION

The main objective of this study was to evaluate the contribution of diet composition and masticatory loading on craniofacial diversification of late Holocene human populations from the southern region of South America. Particularly, we tested whether bite forces, inferred by cross-sectional areas for the major jaw adductors, were associated with variation in size, shape, and form of both the whole skull and the three main functional and developmental modules in which this structure is usually divided. Our results point out that, contrary to the expectation set forth by the biomechanical hypothesis, morphometric variation in cranial traits in the region under study is not correlated with bite forces (Supporting Information Table S1). It is important to remark that the results of this work do not indicate that masticatory loading has no effect on craniofacial morphology, but this was not the most important factor in modeling the pattern of variation among southern South American populations.

These findings might be seen as contradicting previous studies that hypothesized that a reduction in the masticatory muscles' activity and a concomitant decrease in mechanical loading of the skull induce a reduction in muscle size, as well as morphometric changes among human populations (Carlson and van Gerven, 1977; Gonzalez-Jose et al., 2005; Paschetta et al., 2010; von Cramon-Taubadel, 2011). However, most of these studies were based on the assumption that farmers relied on softer diets because of the type of items consumed and the food processing techniques used. The main argument used is that consistency of food produces a mechanical stress that acts differentially on the masticatory complex, so that hunter-gatherers are expected to have

larger facial structures. The approach usually followed to test this hypothesis was to compare morphological traits among populations previously assigned to one broad category that describes its subsistence (e.g., Carlson and van Gerven, 1977; Gonzalez-Jose et al., 2005; Sardi et al., 2006; Paschetta et al., 2010). Nevertheless, differences in masticatory forces were rarely measured directly, and thus this factor was not tested independently from other confounding factors such as food composition. Here, we showed that the magnitude of bite forces and the type of subsistence (farmers vs. hunter-gatherers) is not related in the region under study. Samples from Northwest Argentina, previously described as farmers (Gonzalez Jose et al., 2005; Perez and Monteiro, 2009), exhibited low bite forces, while hunter-gatherers from Patagonia were characterized by a large variation ranging from very low values comparable to those of farmers to high values not found in other samples. This is particularly striking in the sample of hunter-gatherers from Chubut, which displays the lowest BF values despite having large and robust skulls (Fig. 6). Several studies have shown that the use of food processing and cooking techniques, particularly grinding stone tools and pottery, was a widespread strategy across the geographical region during the late Holocene (ca. 2,000 years ago; Bernal et al., 2007, 2010). These practices could have led to the consumption of softer diets, independent of whether the resources were domesticated or not.

We also tested for the association of diet composition and craniometric variation using two independent variables; the CI as a measure of the proportion of carbohydrates consumed and $\delta^{13}C$ values as a measure of the source of proteins. Our results support the hypothesis that variation in overall cranial size and form among populations in the region under study is related to the variation in diet composition. Around 60% of variation in whole cranial size and form was explained by the variable that measure the proportion of carbohydrates consumed. The association between this ecological dimension and morphology was still significant after accounting for the geographic structure of both types of data. Particularly, samples with lower CI display bigger skulls, meaning that diets with a reduced proportion of carbohydrates are associated with skulls of larger size. Conversely, populations that incorporated a higher amount of carbohydrates in their diets have smaller skulls. Experimental and comparative data demonstrate that the consumption of diets with a relatively larger proportion of carbohydrates than proteins induce a decrease in body and skull size with the correlated allometric shape changes (Pucciarelli and Oyhenart, 1987; Stynder et al., 2007; Frisancho, 2009; Stock et al., 2011; Auerbach, 2011). This effect of nutrient intake on bone growth is mediated by the regulation of the GH-IGF axis, which has a central role in stimulating the proliferation and differentiation of chondrocytes and bone formation (Thissen et al., 1991; Bonjour et al., 2001; Nijhout, 2003). Particularly, diets with a lower proportion of proteins impair both the production and action of IGF-I and induce GH resistance that in turn cause growth arrest of skeletal structures.

When the three cranial modules were analyzed separately, we found that size and form variation of face and vault were also associated with CI, although the percentage of variation accounted by this ecological variable was lower than the value obtained for the whole skull.

For the cranial base, the only association found was between size and CI (Supporting Information Table S1). The fact that the size of cranial structures not directly related to masticatory function is also associated with diet composition suggests the effect of systemic factors on skeletal growth. Similarly, a significant relationship between diet and dental size variation among South American populations has been reported in previous studies (Bernal et al., 2010). Populations that relied on diets rich in carbohydrates have the smallest teeth, while terrestrial hunter-gatherers are characterized by larger dental size. In the context of population studies, the association between morphological characteristics and ecological variables is commonly viewed as the result of nonrandom factors such as selection and phenotypic plasticity (Hendry and Kinnison, 1999). Given the recent divergence of groups with markedly different diets (50–100 generations; Harlan, 1971; Pearsall, 1992, 2008; Perez and Monteiro, 2009) and the relatively high population density in the Late Holocene, natural selection does not seem to be the most probable factor behind morphological diversification among these populations. In large populations, natural selection can influence phenotypic changes only when the environmental factors (representing selective pressures) have acted through long periods of time, and thus, several generations have been exposed to them. Selection can act over short time-scales when population size is small and thus adaptive peaks can be reached by chance (Wade and Goodnight, 1998; Hendry and Kinnison, 1999). Conversely, phenotypic plasticity that occurs at the ontogenetic time scale can be though as the most plausible factor driving morphological diversification at short evolutionary time-scales in large populations (Carroll et al., 2007; Perez and Monteiro, 2009).

The results obtained in this work show that 3D craniofacial shape variation among southern South American populations, unlike size and form variation, is independent from the ecological dimensions analyzed here and exhibits a strong spatial structure (as is depicted by correlograms with asymptotically decreasing autocorrelation values; Fig. 5). Consequently, evolutionary neutral or random factors (e.g., genetic drift) may be central for understanding shape variation in the region under study. This is in agreement with the strong adjustment to geographical distance of dental shape and molecular variation observed in the region of study (Moraga et al. 2000; Schurr, 2004; Bernal et al., 2010). Moraga et al. (2000) and Bernal et al. (2010) suggested that the spatial pattern of molecular and morphological shape variation in South America might have resulted either from gene flow restricted by geographical distance (i.e., isolation by distance model; Barbujani, 2000; Hey and Machado, 2003) or from a serial founder effect (Hey and Machado, 2003). The geographical structure of human populations generates more gene flow between nearby populations, leading to high phenotypic and genetic similarities between populations that are geographically close and differences between geographically distant populations by genetic drift. A similar pattern can emerge as the result of the colonization of an area through successive dispersion events of populations with a small number of individuals, which leads to several random sampling or serial founder events (Hey and Machado, 2003). This second process is highly supported by molecular and dental data, which show a linear increase in distances in the main direction of peopling—

that is, from North to South—(Moraga et al., 2000; Bernal et al., 2010; de Saint Pierre et al., 2012).

In summary, the results obtained in this study point out the complexity of the relationship between morphological diversification and environment. Traditionally, studies intended to elucidate the factors driving the evolution of human populations have been conducted under the assumption that variation in craniometric data mainly reflects neutral processes. Even though the effect of environmental factors has been recognized, only in recent years they have been incorporated to the study of human diversification (Roseman, 2004; Gonzalez-Jose et al., 2005; Stynder et al., 2007; Perez et. al., 2011). This article is, to the best of our knowledge, the first work that has systematically quantified two relevant variables associated with diet, masticatory loading, and food composition, for disentangling their contribution to craniofacial variation among populations at short temporal scales. Our results suggest that food production increased the availability of carbohydrate enriched diets creating novel environmental conditions that in turn contributed to changes in morphology among populations from southern South America. Further studies that explicitly incorporate quantitative data of multiple ecological dimensions into the analyses will continue to shed light on the factors that shape morphological changes at different temporal and geographic scales and environmental settings in human populations.

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