Ecological and evolutionary factors in dental morphological diversification among modern human populations from southern South America

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The knowledge of processes involved in morphological variation requires the integrated analysis of evolutionary and ecological factors. Here, we investigate the factors responsible for dental variation among human populations from southern South America. The aim of this work is to test the correspondence of dental size and shape variation with geographical, molecular (i.e. mtDNA) and ecological (i.e. climate, diet and food preparation) variables employing comparative phylogenetic methods, which have not previously been extensively applied at a within-species level. The results of the Procrustes analysis show a significant association of shape variables with molecular distance and geography, whereas dental size is not associated with molecular or geographical distances among groups. Phylogenetic generalized least-squares analysis, which takes into account the evolutionary autocorrelation among populations, shows a significant relationship between dental size variation and diet, while temperature and pottery do not correspond with dental size or shape. Specifically, groups with diets rich in carbohydrates, as well as the maritime hunter-gatherers, have the smallest teeth. In summary, our results support ecological factors as the dominant factor on dental size diversification in this region, while evolutionary relationships account for variation in dental shape.

Keywords: dental size and shape; phylogenetic comparative method; temperature; diet; pottery

1. INTRODUCTION

Morphological variation within and between modern human populations is the result of evolutionary history and ecological factors acting over time. Heritable variation patterns are mainly owing to the differential contribution of processes such as drift, gene flow and natural selection, and historical events, such as population expansion and extinction or the founder effect (Cavalli-Sforza *et al.* 1994; Templeton 2007). In addition, phenotypic differences could arise from environmental influences during ontogeny (e.g. phenotypic plasticity; Carroll *et al.* 2007). The study of these factors is highly complex and requires the consideration of multiple dimensions, such as the evolutionary relationships estimated by neutral molecular data, ecological diversity described as biotic and abiotic variables, and morphological variation measured with morphometric techniques (Schluter 2000).

Southern South America is a region of great interest for understanding processes of morphological diversification, owing to the availability of a large amount of data on evolutionary relationships and ecological variables. It was also one of the last colonized regions of the planet, being colonized by small groups of hunter–gatherers about 11 000–13 000 BP (Borrero 1999). Molecular analyses based on D-loop mtDNA sequences, frequencies of mtDNA haplogroups and Y-STR sequences support a common origin for all South American populations and suggest that differentiation was the result of a founder effect, which occurred during the initial peopling of the southern cone of the continent (García-Bour *et al.* 2004). These populations occupied a wide range of environments with large differences in mean annual temperature (from 21° to 4°)—and consequently in available resources—spread along 3500 km. This ecological variation was augmented during the last 3000 years by food production (i.e. agriculture) and food preparation technology (i.e. pottery and grinding tools). Agriculture made carbohydrates more available to farming groups, in contrast to the relatively large proportion of protein consumed by hunter–gatherer groups (Berberián & Nielsen 2001).

In this study, we investigate the factors responsible for dental variation in human populations from southern South America. Teeth exhibit particular genetic and developmental characteristics. Previous studies indicate that human teeth display moderate to high heritability values, ranging from 0.5 to 0.9 depending on the tooth class and variable analysed (Dempsey & Townsend 2001; Townsend *et al.* in press). Developmentally, teeth grow inside follicles until their morphological formation

is complete. Therefore, environmental influence is limited to the early ontogenetic stages of individuals (Hillson 1996). Consequently, it has been suggested that teeth represent their evolutionary history with greater accuracy than other skeletal structures (Sperber 2004). Morphometric studies that have analysed dental variation across human populations assume that dental shape and size variation is related either to random processes, such as genetic drift and mutation, or non-random factors, such as natural selection and phenotypic plasticity, but they rarely assess the contribution of phylogenetic and ecological factors (Kieser 1990; Harris & Rathburn 1991; Hillson 1996; Schnutenhaus & Rösing 1998; Hanihara & Ishida 2005; among others).

The aim of this work is to test the correspondence of dental size and shape variation among populations from southern South America with geographical, molecular and ecological (i.e. climate, diet and food preparation) variables using comparative methods (i.e. comparative phylogenetic methods; Diniz-Filho 2000; Rohlf 2001; Garland *et al.* 2005). Comparative phylogenetic methods allow us to infer the influence of ecological factors while considering non-independence among samples owing to their common evolutionary history. Despite the fact that these new methods have undergone rapid development and have been applied to many morphological studies at the interspecific level (see Garland *et al.* 2005), relatively little work at the intraspecific level has been performed to date (Hansen *et al.* 2000; Felsenstein 2002).

2. MATERIAL AND METHODS

(a) *Samples*

We analysed permanent teeth from male and female adults belonging to 12 archaeological samples from southern South America: Calchaqui Valley (CV, $n = 16$) and San Juan (SJ, $n = 23$) in northwestern Argentina; Chaco (Cha, $n = 14$); Delta (Del, $n = 21$) and Pampa (Pa, $n = 37$) from the Pampean Region; Araucania (Ar, $n = 18$) from the Araucanian Region; Neuquén (Nqn, $n = 17$), Chubut Valley (ChV, $n = 26$) and South Patagonia (SP, $n = 15$) from continental Patagonia; and Tierra del Fuego (TF, $n = 22$), Austral Island (AI, $n = 20$) and Beagle Channel (BC, $n = 13$) from insular Patagonia. All samples were assigned to the final late Holocene (*ca* 1500–500 years 14C BP) based on radiocarbon dating and contextual information (Bernal 2008). The samples come from groups that inhabited different geographical and ecological regions and practiced different subsistence systems (see table S1 and figure S1 in the electronic supplementary material).

Sex and age estimations were made using cranial and pelvic features (Buikstra & Ubelaker 1994). Because there is no sexual dimorphism in tooth shape and almost all samples were sex-balanced, males and females were pooled in the following analyses to obtain greater sample sizes. However, two samples showed sexual dimorphism in tooth size and three samples were not sex-balanced. Unbalanced samples could generate biased results in the analyses of size variation, so we repeated such analyses using male individuals only (see below).

(b) *Morphometric and statistical analyses*

Morphometric data for all upper (U) teeth were collected, with the exception of third molars. Teeth that were not completely erupted, obscured by crowding, presented carious lesions or exhibited severe wear affecting the cervix were excluded from the analysis. Measurements of the left teeth were used for statistical analyses, but when the left measurement was missing, data from the right antimere was substituted. Morphometric dental variation was analysed with mesiodistal (MD) and buccolingual (BL) diameters measured at the base of the crown along the cement–enamel junction, using a Mitutoyo Digimatic calliper with thin points: Paleo Tech Hillson/Fitzgerald Dental Caliper (Hillson *et al.* 2005; Bernal 2008). Despite the common usage of maximum crown diameters, these alternative dental measurements can be measured just as reliably, record similar information about tooth crown size, and are better measures for worn dentitions (Hillson *et al.* 2005; Stojanowski 2007). Because all measurements of anterior dentition had a large amount of missing data, only the upper premolars (UPM3 and UPM4) and molars (UM1 and UM2) were included. To control for inter-observer error, all dental measurements used in this study were recorded by V.B. The intra-observer error was controlled with the experimental design of Bernal (2008). The results indicate that the measurement procedures did not generate significant observational error (Bernal 2008).

The original variables were used to calculate shape and size variables. The geometric mean (the *n*th root of the product of all *n* variables) was used as a general tooth size measure (Jungers *et al.* 1995). Shape variables were calculated by dividing each measurement by the geometric mean for each individual in the sample. This procedure generates Mossiman shape variables (Jungers *et al.* 1995). Then, mean values of shape variables were used to perform a principal component (PC) analysis on the covariance matrix. This analysis describes the major trends in tooth shape variation among samples. PCs calculated with mean values of shape variables give a robust description of shape differences among samples because the small sample sizes and heterogeneous covariance structures between samples have little influence on this analysis (Polly 2003). In addition, PCs are low-dimensional axes of a Euclidean space that measures morphological differences, whereas other distances, such as Mahalanobis distances, measure statistical distinctness of two groups.

We performed a Procrustes analysis (Peres-Neto & Jackson 2001) to test the correspondence of the ordination of size (i.e. geometric mean) and shape variation (i.e. PC scores accounting for 90% of total variation) with molecular (i.e. mtDNA haplogroup frequencies) and geographical variation (see table S2 in the electronic supplementary material). In particular, the ordination of the size vector and shape variables (PC vector of mean values for each sample) was compared with the principal coordinates from the distance matrix based on mtDNA haplogroups. We also performed the Procrustes analysis using shape and size variation with geographical coordinates of the samples. Because several works have proposed a geographical structure of evolutionary relationships in southern South America (e.g. Moraga *et al.* 2000), this procedure allowed us to confirm the results from ordination of evolutionary relationships calculated with mtDNA data (table 1; for a similar approach see Hansen *et al.* 2000; Felsenstein 2002). The Procrustes method scales and rotates the ordinations using a minimum squared differences criterion. Then, the complement of the sum of the squared residuals between configurations in their optimal superimposition can be used as a measure of

Table 1. Procrustes analyses comparing the ordinations obtained with dental size (geometric mean) and shape (Euclidean distances) to molecular and geographical distances. (Bold numerals represent statistically significant values.)

comparisons	m_{12}	p
molecular versus geographical distances	**0.7147**	**0.001**
molecular distance versus size	0.2547	0.538
geographical distance versus size	0.1782	0.643
molecular versus Euclidean distances	**0.5608**	**0.047**
geographical versus Euclidean distances	**0.5630**	**0.010**
size versus Euclidean distance	0.2164	0.743

association ($m_{12} = \sqrt{1 - SS}$). A permutation procedure (PROTEST; 10 000 permutations) was used to assess the statistical significance of the Procrustean fit (Peres-Neto & Jackson 2001). The Procrustes test for shape variation was significant (table 1; see below). Thus, to take into account evolutionary and/or spatial autocorrelation, evolutionary relationships and spatial distance among southern South American groups were included in a subsequent analysis (Garland *et al.* 2005; Freckleton & Jetz 2009).

We evaluated the concordance between shape and size variables with ecological diversity (i.e. climate, diet and food preparation techniques; see table S1 in the electronic supplementary material) using a Phylogenetic generalized least-squares (PGLS) analysis (in comparative method; Martins & Hansen 1997; Rohlf 2001). The mean annual temperature where populations were located was used as an indirect estimator of climate (Katzmarzyk & Leonard 1998). This variable was obtained from climatic databases (http://www.smn.gov.ar/). We also defined dummy variables to describe diet differences between populations. These variables include four categories: terrestrial hunter–gatherer groups with a diet mainly based on terrestrial faunal resources (ChV, SP, TF); maritime hunter–gatherers with a diet mainly based on marine resources (AI, BC); and two groups with differing reliance on domesticated resources, farmers (VC, SJ) and horticulturists (Pa, Ar, Nqn). In addition, we evaluated diversity in food preparation techniques through one variable that describes the time depth of pottery use, absent in southernmost populations (SP, TF, AI, BC, see table S1 in the electronic supplementary material). The use of grinding stone tools was not included as a variable because during the late Holocene it spread across every geographical region analysed. We fitted the ecological variation (temperature, diet and pottery) to shape (the PCs 1, 2 and 3) and size scores using the usual linear regression model:

$$\mathbf{P} = \mathbf{XB} + \varepsilon,$$

where **P** is the shape or size scores matrix; **X** represents a matrix containing mean annual temperatures to describe climatic variation, the values of dummy variables used to define diet membership and/or food preparation techniques; **B** is the matrix of partial regression coefficients and ε is the error term. To account for evolutionary non-independence, PGLS assumes that ε has a covariance matrix (**C**) derived from the evolutionary relationships among groups (Martins & Hansen 1997; Rohlf 2001). We used two covariance matrices based on the Brownian model. The first matrix was based on
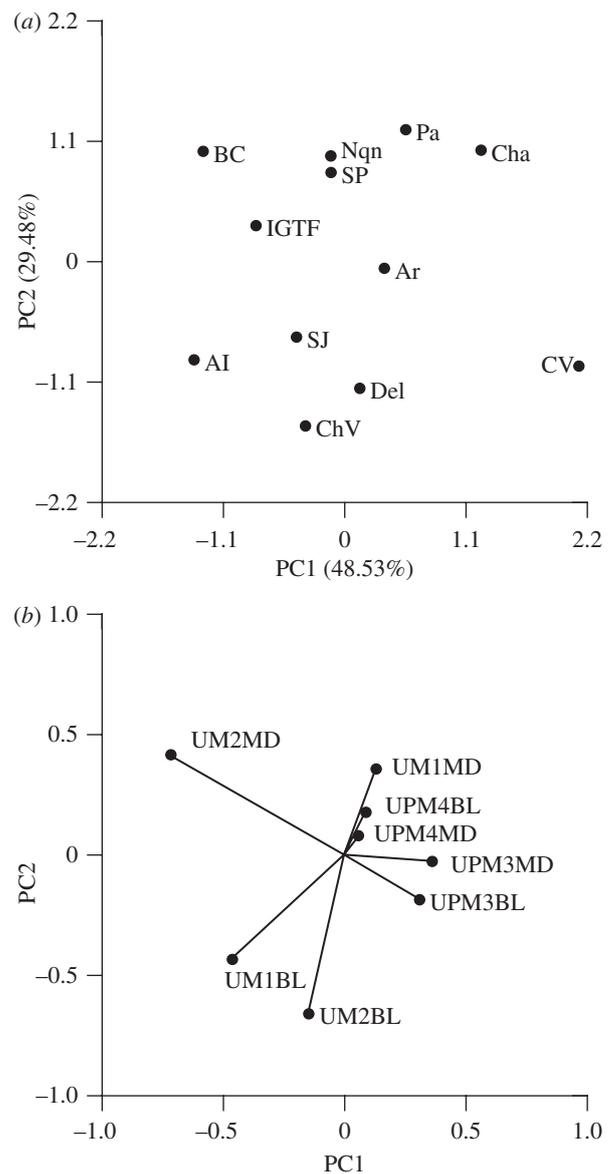

Figure 1. (*a*) Principal component (PC) analysis performed on dental shape variables. (*b*) Plot of the variables for PC1 versus PC2.

a neighbour-joining tree, and assumes independent evolution of the populations after the initial divergence (see Rohlf 2001 for a detailed description of how an evolutionary tree can be used to construct the expected covariance matrix for the taxa). The second matrix was calculated as the inverse function of mtDNA Euclidean distances between groups, $c_{ij} = 1/d_{ij}^2$, where d is the Euclidean distance between two groups. This matrix assumes a model of spatial structuring in the evolutionary relationship among samples, generated by isolation by distance (IBD). When a covariance matrix based on the inverse function of geographical distance was used, the results did not change. The significance of the regression model was assessed by the *F*-statistic.

Statistical analyses were performed using R v. 2.8.1 (R Development Core Team 2008).

3. RESULTS
The ordinations of population means calculated from the shape variables are shown in figure 1*a*. The first two PCs explain approximately 78 per cent of the total variation.

Table 2. Regression analyses of dental size and shape variation on diet and temperature using PGLS. (Bold numerals represent statistically significant values.)

comparisons	Wilks' lambda	R^2	F	p
size versus diet		**0.682**	**5.716**	**0.021**
size versus temperature		0.008	0.084	0.777
size versus pottery		0.029	0.300	0.596
size versus diet + temperature + pottery		0.684	3.792	0.060
shape versus diet	0.284		1.108	0.414
shape versus temperature	0.328		1.986	0.128
shape versus pottery	0.386		1.627	0.204
shape versus diet + temperature + pottery	0.107		2.053	0.120

These PCs show a general geographical ordination. Along the first PC axis UM2MD and UM1BL have the highest and negative values of component loading (figure 1*b*), whereas UPM3MD has a high and positive value (figure 1*b*). Along the second axis, the variables with larger component loading are UM2BL and UM1BL (figure 1*b*), whereas the contribution of premolars is negligible. Size variation among samples shows that terrestrial hunter–gatherers have a larger tooth size than maritime hunter–gatherers, farmers and horticulturists (see table S1 in the electronic supplementary material). The male-only analyses show the same pattern (results not shown). Moreover, the mean size for male samples was significantly correlated with the mean size for males and females ($r = 0.91$; $p < 0.01$).

The Procrustes analysis of ordinations based on mtDNA haplogroup frequencies, geographical coordinates and dental variation shows a significant association among shape variation (i.e. 90% of PC variation), molecular distance and geography (table 1). Dental size, however, is not associated with molecular or geographical distances among groups (table 1). Independent regression of ecological variables using PGLS—employing the inverse function of mtDNA distance as the **C** matrix—shows a significant relationship between dental size and diet, explaining 68 per cent of size variation (table 2). Size and shape variables do not correspond with temperature or the time depth of pottery use (table 2). Taking these results into account, we tested whether evolutionary relationships and diet are independent, and we found a lack of association between these variables ($m_{12} = 0.318$; $p = 0.759$). F-tests of the model that includes the three ecological variables (i.e. temperature, diet and pottery) revealed a marginally significant value (table 2). This model explains the same percentage of size variation as the diet-only regression model (68%). The regression analysis performed with a **C** matrix based on a neighbour-joining tree shows similar results (results not shown).

4. DISCUSSION AND CONCLUSION

Our methodological approach, based on Procrustes and PGLS tests, allows us to estimate the correspondence between morphological variation and evolutionary relationships. We were able to estimate the influence of ecological factors while taking into account the fact that more closely related populations tend to resemble one another as a result of either gene flow or common ancestry (Felsentein 2002). Among available comparative phylogenetic methods, PGLS was chosen because it is a robust tool that can apply several models of trait evolution, such as Brownian motion or Ornstein-Uhlenbeck processes (Rohlf 2001; Garland *et al.* 2005). In our study, the problem of non-independence of populations has been solved using a Brownian motion model to generate several covariance matrices for the ε term, which were then incorporated into the PGLS model. We generated three weight matrices using common evolution along a neighbour-joining tree (Rohlf 2001; Garland *et al.* 2005), the inverse of mtDNA distance and the inverse of geographical distance. This is a broad approach that solves the problem of spatial structuring in biological variation (generated by serial founder effects or an IBD model). This approach is relevant to studying the importance of evolutionary history and ecological factors in the diversification of extant and prehistoric populations.

The results of the Procrustes test suggest that evolutionary history is the dominant factor driving dental shape diversification among human populations in southern South America (table 1). The lack of significant correspondence between dental shape variation and ecological variables (table 2) is further evidence against ecological variation as the main factor behind dental shape divergence in these populations.

The existence of spatial structure in dental shape variation in Euclidean space, shown by association between geographical coordinates and PCs of shape variables, agrees with previous studies indicating that the Mahalanobis distance between samples shows the greatest adjustment to geographical distance in the region under study (Bernal 2008; Bernal *et al.* in press). This pattern corresponds with molecular data showing that the evolutionary distance between samples from southern South America has a clear association with geographical distance (Moraga *et al.* 2000; Schurr 2004).

Consequently, evolutionary historical factors are central in accounting for dental shape variation in the region under study. Bernal (2008) and Bernal *et al.* (in press) suggested two factors that could explain this pattern of shape variation. First, this pattern could emerge as a result of gene flow restricted by geographical distance (i.e. model of IBD), or second, by serial founder effects. Because of the spatial structure of human populations (Cavalli-Sforza *et al.* 1994), gene flow will occur more frequently between nearby populations, leading to high genetic affinities between groups that are geographically close and the probable genetic differentiation of geographically distant groups by genetic drift (i.e. the IBD model; Cavalli-Sforza *et al.*1994). On the other hand, the increase in biological distance with geographical distance could be the result of the colonization of an inhabited area through multiple, successive dispersion events of groups with a small number of individuals, a process known as range expansion (Slatkin 1993). Range expansion also leads to several random sampling events, serial founder events, resulting in a within-population reduction gradient of biological diversity

away from the centre of expansion, unless rates of migration are extremely high (Ramachandran *et al.* 2005). This hypothesis is supported by recent molecular data, which show a pattern of decreasing genetic variation in the main direction of peopling—i.e. from north to south (Moraga *et al.* 2000; García-Bour *et al.* 2004). The results of the haplogroup frequency analysis indicate that the A and B haplogroups decrease and the C and D haplogroups tend to increase in this direction (Moraga *et al.* 2000).

By contrast, the Procrustes test suggests that evolutionary history is not the dominant factor behind the diversification of dental size among human populations from southern South America. PGLS tests support ecological factors, in particular diet variation, as the dominant driver of size diversification in this region. In particular, the groups with diets rich in carbohydrates (farmers and horticulturalists) and the maritime hunter–gatherers have the smallest teeth. This suggests the importance of diet, or some diet-related variable, on dental size variation. The differences we found between size and shape indicate that these components of dental variation might change independently. Moreover, the correlation between geometric mean and PC1 scores is 0.369 ($p = 0.238$), i.e. the influence of size on shape variation among populations is weak. Such results contrast with the allometric change documented for cranial structures in the same region (Perez & Monteiro 2009).

Although dental size reduction is well documented for several populations of *Homo sapiens*, the factors underlying this phenomenon are still controversial (Brace & Mahler 1971; Lukacs 1985; Brace *et al.* 1987; Calcagno & Gibson 1988; Sciulli *et al.* 1988). For instance, Calcagno & Gibson (1988) proposed that shifts to a softer and/or more cariogenic diet resulted in selection for smaller teeth. Other authors have postulated that the consumption of softer food owing to the development of cooking and pottery resulted in a relaxation of selective pressures for large teeth and a concomitant accumulation of random mutations, with an overall effect of structural reduction (Brace *et al.* 1987). Such accumulation of random mutations might arguably have caused dental reduction in isolated, small hunter–gatherer groups where the influence of genetic drift is significant, but it is unlikely in societies with greater populations and gene flow, as is the case with the farmers and horticulturists analysed here. In addition, dental size, similar to corporal and cranial dimensions, can also be influenced through ontogeny by environmental conditions such as malnutrition and disease (Guagliardo 1982). Particularly in the region we studied, the strong relationship between size and diet suggests the influence of non-random factors such as selection or phenotypic plasticity as probable causes of dental size variation.

There are significant dietary differences in the late-Holocene humans of southern South America; including terrestrial and maritime hunter–gatherers, as well as populations surviving on a variable percentage of cultigens (Berberián & Nielsen 2001). Thus, these populations varied widely in their intake of proteins, carbohydrates and micronutrients. It is widely recognized that agricultural practices have had a profound impact on human health and lifestyle, such as providing greater availability of carbohydrates as well as reduced access to key micronutrients, compared with protein-based diets of hunter–gatherer groups (Larsen 2006). Because environmental influences during ontogeny could not be controlled in our study, the genetic and ecophenotypic components of morphological change could not be isolated (Hendry & Kinnison 1999; Carroll *et al.* 2007). However, the recent ecological divergence between groups (agriculture emerged 50 or 100 generations BP), suggests the importance of phenotypic plasticity in this region (Carroll *et al.* 2007). Phenotypic plasticity has been hypothesized to account for facial shape and cranial size differences in this region (Perez & Monteiro 2009), suggesting the systemic influence of non-random factors. Further studies investigating this hypothesis and its alternatives (e.g. directional selection) are needed to elucidate factors contributing to size differences among southern South American populations.

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