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Association between shape changes and bone remodeling patterns in the middle face during ontogeny in South American populations

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Abstract

The morphology of facial bones is modeled by processes of bone formation and resorption induced by interactions between tissues and compensatory responses. However, the role of remodeling patterns on the morphological changes within and among populations has been scarcely explored. Here, we assess the association between facial shape and the underlying bone cell activity throughout the ontogeny in two Amerindian populations that represent the extremes of craniofacial variation in South America. The sample comprises 71 individuals (36 adults and 35 subadults) representing hunter-gatherers from Patagonia and horticulturists from Northwest Argentina. We analyzed the shape and size of the zygomatic and the maxilla, and compared them with the patterns of bone formation and resorption. Bone formation and resorption were described by quantitative histological analysis of bone surfaces. Morphological changes were described by landmarks and semilandmarks digitized on 3D surfaces obtained from CT images. The results from multivariate statistics analysis show that the patterns of bone remodeling are associated with variation in the morphology of the middle face. We found a similar pattern of facial shape variation along the ontogenetic trajectory in the two samples, and a similar trend in the amount of formation and resorption activities across ages. The main differences between samples were found in the distribution of the areas of bone formation and resorption, possibly associated with mechanical bone response to masticatory loading. These findings provide clues about the processes and mechanisms of bone development involved in the facial morphological differentiation in human populations from southern South America.

KEYWORDS

bone formation and resorption, geometric morphometrics, partial least squares

1 | INTRODUCTION

Craniofacial morphology results from developmental processes, which are in turn driven by ecological and evolutionary factors that shape the pattern of variation among populations (Gonzalez, Perez, & Bernal, 2011; Klingenberg, 2010; Strand Viðarsdóttir, O'Higgins, & Stringer, 2002). Throughout individual ontogeny, the craniofacial skeleton is under the effect of mechanical forces associated with the interactions among bones and with other tissues and organs (e.g., muscles, teeth, brain), as well as the effect of molecular signaling (Enlow & Hans, 1996; Martin, Burr, Sharkey, & Fyhrie, 2015; Moss & Young, 1960). Altogether, these interactions and compensatory responses stimulate or inhibit cell differentiation in osteoblasts and osteoclasts involved in the process of bone formation and resorption that constitute bone growth remodeling (Enlow, 1963; Enlow & Hans, 1996). They also cause passive movements that is, growth displacements—that occur in response to the movement of adjacent tissues (Enlow & Hans, 1996). Accordingly, bone remodeling keeps the spatial proportion and functional relation of the bone during craniofacial growth, balancing the displacements, and thereby patterning the bone shape. Therefore, the distribution of remodeling activity observed on surfaces is considered to be an important indicator of the growth pattern of craniofacial structures (Bromage, 1982; Enlow & Hans, 1996; O'Higgins & Jones, 1998). Spatial and temporal changes in the activity of osteoclasts and osteoblasts shape cranial growth and development during the ontogeny, resulting in size, and shape differences among individuals and populations (Brachetta-Aporta, Gonzalez, & Bernal, 2019a, 2019b; Brachetta-Aporta, Martinez-Maza, Gonzalez, & Bernal, 2014; Freidline, Martinez-Maza, Gunz, & Hublin, 2017; Martínez-Vargas, Muñoz-Muñoz, Martinez-Maza, Molinero, & Ventura, 2017; Schuh et al., 2020).

Previous studies show that bone remodeling patterns differ between adults and subadults, which indicates that growth dynamics changes through ontogeny (Brachetta-Aporta et al., 2014, 2019a; Kranioti et al., 2009; Martinez-Maza, Rosas, & Nieto-Diaz, 2013; McCollum, 2001, 2008). Particularly, the middle face—that is, maxilla and zygomatic bones—goes under significant changes during postnatal ontogeny, unlike the upper face that seems to be more constant with age and also among populations (Brachetta-Aporta et al., 2019a). When the deciduous teeth are developing, the bone surface of the anterior maxilla is characterized by bone formation, followed by a predominance of bone resorption between 2 and 14 years-old, although the extension and distribution of the areas varies among samples (Enlow & Bang, 1965; Kurihara, Enlow, & Rangel, 1980; Martinez-Maza et al., 2013; McCollum, 2008). On the other hand, the zygomatic of subadults exhibits large areas of bone deposition (Brachetta-Aporta et al., 2019a; Enlow & Bang, 1965; Martinez-Maza et al., 2013). The few studies on the maxilla and the zygomatic in adults indicate a higher proportion of formation, with more limited areas of resorption around the insertion of the masseter in the zygomatic and in the canine fossa and the alveolar process of the maxilla (Brachetta-Aporta et al., 2014, 2019a; Martinez-Maza et al., 2013). Such differences in the bone remodeling patterns suggest that there are changes in the morphological configuration of the middle face during growth trajectory (Brachetta-Aporta et al., 2019b; Schuh et al., 2020), which in turn would have a key role in population differentiation (Freidline, Gunz, & Hublin, 2015; Gonzalez et al., 2011; Strand Viðarsdóttir et al., 2002).

To which extent the changes in the remodeling patterns of the middle face are related with morphological changes within and among populations has been scarcely explored (Brachetta-Aporta et al., 2014; Freidline et al., 2017; Martinez-Maza et al., 2013; McCollum, 2008). Recent studies have assessed the association between bone remodeling maps and morphometric variation, although they are limited to the maxilla (Brachetta-Aporta et al., 2019b; Schuh et al., 2020; Schuh, Kupczik, Gunz, Hublin, & Freidline, 2019). In a sample from South America the presence of bone formation was found to be associated with the increase in size throughout ontogeny, and with a greater inferior projection of the maxilla in adults; while bone resorption was associated with an inferior displacement of the bone in subadults (Brachetta-Aporta et al., 2019b). This characterization corresponds to a prehistoric population with a diet based on domestic and wild plants and animals, while it remains to be evaluated if the same applies to other human populations.

The aim of this work is to assess the association between bone remodeling patterns and the changes in the morphology of the middle face throughout ontogeny in two Amerindian populations from southern South America. The samples selected are at the extremes of craniofacial variation in the region and represent populations with different subsistence strategies, that is, hunter-gatherers and horticulturalists. Previous studies suggested that environmental factors, and particularly diet, played a significant role in the morphological diversification of South American populations (Barbeito-Andrés, Pucciarelli, & Sardi, 2011; Bernal et al., 2014; Bernal, Perez, Gonzalez, & Felizola Diniz-Filho, 2010; Gonzalez et al., 2011; Gonzalez, Perez, & Bernal, 2010; González-José et al., 2005; Menéndez, Bernal, Novellino, & Perez, 2014; Paschetta et al., 2010; Perez et al., 2011; Perez & Monteiro, 2009; Sardi, Novellino, & Pucciarelli, 2006). Consequently, we expect differences in the distribution and extension of areas of bone formation and resorption associated with the morphological changes of the facial skeleton across ontogeny in the two samples. The results will contribute to the discussion of the growth processes and mechanisms involved in the differentiation of craniofacial morphology within and between populations.

2 | MATERIALS AND METHODS

2.1 | Sample

The osteological sample comprises 71 individuals (36 adults and 35 subadults) from Pampa Grande in north-western Argentina (PG; 15 subadults and 17 adults) and the lower valley of the Chubut river in Argentine Patagonia (20 subadults and 19 adults). The individuals from Pampa Grande are characterized by small skulls with graceful traits, and have been assigned to horticulturist groups that inhabited the region around 1720 ± 50 years BP. In contrast, the individuals from Chubut belong to hunter-gatherers from Patagonia dated between 2,600 and 200 years BP and are characterized by robust skulls with a marked development of supraorbital and superciliary arches. Morphological differences between both samples have been associated to the higher levels of force exerted during chewing related to more abrasive and hard diets in the huther-gatherer group (Baffi, Torres, & Cocilovo, 1996; Barbeito-Andrés et al., 2011; Bernal, Perez, & Gonzalez, 2006; Gonzalez et al., 2010; Lema, 2011; Menéndez et al., 2014; Perez, Bernal, & Gonzalez, 2007; Sardi, Ramírez-Rozzi, González-José, & Pucciarelli, 2005). For a more detailed description of both samples, see the Supporting Information. The materials are deposited in the Anthropology Division of Museo de La Plata (Buenos Aires, Argentina).

The individuals were previously selected on the basis of their preservation (Brachetta-Aporta et al., 2014, 2019a). Only skulls with less than 25% of postdeposition bone loss (Buikstra & Ubelaker, 1994) and a low weathering stage (≤1; Behrensmeyer, 1978) were included. Age and sex were estimated on the basis of morphological traits. The degree of the dental development and eruption in subadults was assessed on CT-scans using standard references (AlQahtani, Hector, & Liversidge, 2010; Buikstra & Ubelaker, 1994). Subadults were grouped in four categories based on a formation score for the permanent teeth (Brachetta-Aporta and D'Addona, 2020): G1: up to 4.4 year-old; G2: from 4.5 to 10.4 year-old; G3: from 10.5 to 14.4 year-old; G4: from 14.5 to 18 year-old. Adults were assigned to two age categories according to the degree of obliteration of the sphenobasilar and ectocranial suture (Meindl & Lovejoy, 1985): G5: Young Adult (20–34 year-old); G6: Middle-aged Adult (35–49 year-old). Sex was estimated for adults using the degree of development of the glabella, supraorbital margin, mastoid process, supramastoid crest, and nuchal crest (Buikstra & Ubelaker, 1994).

2.2 | Morphometric analyses

A CT-scan of each skull was acquired at CIMED and Mon diagnosis imaging centers from La Plata, Argentina. The resolution of the CT images was 1,024 × 1,024 (pixel size: 0.165 × 0.165 mm; slice thickness: 0.33 mm) and 512 × 512 (pixel size: 0.345 × 0.345 mm, slice thickness: 0.33 mm), respectively. The CT images were converted from DICOM format to 3D models, with nearly equal number of vertices and faces, and saved in .PLY format. A set of 50 landmarks and 187 semilandmarks along facial contours and surfaces were digitized on the 3D digital models in .PLY format (Figure 1). Landmarks and curve semilandmarks were digitized manually in Avizo 8.0 in order to guarantee their location, while, the surface semilandmarks were digitized by a semiautomatic protocol (Gunz & Mitteroecker, 2013) implemented in geomorph and Morpho packages for R (R Core Team, 2014). To remove non shape variation, a Generalized Procrustes Analysis (GPA) was performed (Rohlf & Slice, 1990). Additionally, semilandmarks were slid by thin-plate spline (TPS) minimizing the bending energy between the target and reference configurations (Klingenberg, 2013; Mitteroecker & Gunz, 2009). Semilandmarks digitized on curves were slid along tangents while surface semilandmarks were slid along the tangent plane (Gunz & Mitteroecker, 2013). The intraobserver error associated with the placement of landmarks and semilandmarks was previously evaluated (Brachetta-Aporta, 2018). Briefly, one of us (N. B. A.) obtained two sets of coordinates in 15 skulls that were compared by a correlation coefficient and ANOVA tests. The results indicate low levels of measurement error for the coordinates of points digitized here ($cc = 0.98$, $p = .341$). For handling missing data while preserving the largest number of individuals, the landmarks and semilandmarks over damaged surfaces were replaced through the imputation of missing values by bending energy (Gunz, Mitteroecker, Neubauer, Gerhard, & Bookstein, 2009; Nesser, 2007). The imputation procedure was performed in the geomorph package for R (R Core Team, 2014). The resulting Procrustes shape coordinates were extracted, and the centroid size (CSz) obtained from the GPA was used as a measure of size (Bookstein, 1991).

Size-related shape changes or allometric changes along the ontogenetic trajectory of each sample were analyzed by a multivariate regression analysis. Superimposed coordinates were used as the dependent variables and the centroid size as the independent variable (Monteiro, 1999), where the dependent variable accounts for shape changes per unit of size increase (i.e., regression score shape; Drake & Klingenberg, 2008). Shape changes associated with size were represented by

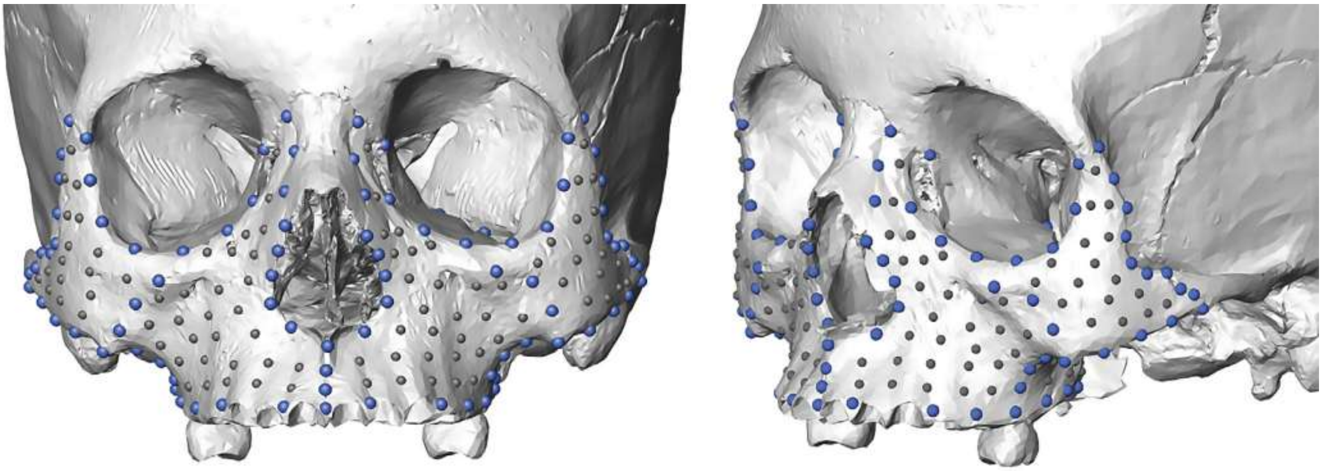

FIGURE 1 Landmarks and semilandmarks of contours (blue) and semilandmarks of surfaces (black) digitized on the facial skeleton

morphings (Klingenberg, 2013). Additionally, the allometric trajectories of both samples were compared by a linear model including the shape coordinates as dependent variables, whereas size, group and their interaction were included as independent variables for the procD.lm function in the geomorph package (Adams & Otarola-Castillo, 2013). The adult size achieved was compared by an ANOVA test. The analyses were carried out in MorphoJ (Klingenberg, 2011) and R (R Core Team, 2014).

2.3 | Bone surface analysis

The distribution of bone formation and resorption activity of the zygomatic and maxilla bones was analyzed by casts of periosteal bone surfaces as described in Brachetta-Aporta et al. (2019a). To avoid the effect of factors other than bone remodeling on the bone surfaces analyzed, we excluded individuals with taphonomic alterations, pathologies, alveolar resorption due to ante-mortem tooth loss, trauma or abscesses, among others. The casts were made from silicon impressions (i.e., negative casts) using epoxy resin and then covered by a thin layer of gold and palladium to give electric conductivity. The replicas of bone surfaces were observed under an optical microscope Olympus CX31 (20X NA 0.40 objective) with incident light. To facilitate the recording of microstructural features under the microscope, a grid of 5 × 5 mm was drawn on each cast and the information was summarized in color maps. In order to assess bone remodeling activity (i.e., the last bone remodeling activities preserved), we registered the presence of collagen fibers bundles and concavities known as Howship's lacunae, which are associated to forming and resorbing surfaces, respectively (Boyde, 1972; Bromage, 1984; Martinez-Maza, Rosas, & Nieto-Diaz, 2010). Subsequently, bone remodeling was quantified following Brachetta-Aporta, Gonzalez, and Bernal (2018) and missing data were estimated by spatial interpolation and/or imputation (Brachetta-Aporta et al., 2019a). Briefly, the protocol consists in generating a consensus configuration for each anatomical region by means of a Procrustes superimposition of the digitized maps, then a TPS deformation of each map to the consensus is applied in order to them make comparable (Brachetta-Aporta et al., 2018). A new grid is applied to the maps transformed by the previous procedure, which is used to obtain information about the spatial distribution of formation and resorption activities and estimate the missing values. The size of the cells was defined by testing the accuracy of the interpolations obtained, and was set in 10 × 10 pixels for the zygomatic and 15 × 15 pixels for the maxilla. Then, a general bone remodeling map was obtained for subadults and adults of PG and Chubut samples separately. The general maps obtained by this procedure represent the median value of each cell for the age categories. Individual maps with more than 75% of bone remodeling data missing were not included in this estimation. Subadults and adults were analyzed separately because of the differences in shape and size for both regions.

Additionally, we computed the average of bone formation and resorption for each age category from the maps with more than 25% of information. A percentage of bone formation and resorption was obtained in relation to the percentage of cells for which the type of activity was recorded. The results were summarized in bar plots.

2.4 | Comparative analysis of facial morphology and bone remodeling data

Following Brachetta-Aporta et al. (2019b) we evaluated the association between morphological changes and bone remodeling activity of the zygomatic and the maxilla bones by means of a partial least squares (PLS) analysis. The procedure was done using the Procrustes coordinates for each bone (first block) and the principal components (PCs) that accounted for 80% of variation in bone remodeling patterns (second block) developed from the PCA performing with the remodeling information obtained from the complete maps. The PLS analysis computes a linear regression that describes the highest mutual covariance between two or more blocks of variables (Abdi, 2010; Bookstein, Sampson, Streissguth, & Barr, 1990; Rohlf & Corti, 2000). The two sets of variables are treated symmetrically and the resulting coefficients represent the strength of the co-variation between the two blocks (Rohlf & Corti, 2000). The analyses were run in MorphoJ (Klingenberg, 2011) and geomorph package.

3 | RESULTS

3.1 | Patterns of morphological and bone remodeling variation during ontogeny

Figure 2 shows the facial shape changes across subadults and adults from Pampa Grande and Chubut. For Pampa Grande (Figure 2a), 26.75% (p <.0001) of the total variation in shape is explained by changes in size (or allometric changes). The facial morphology changes to a straight and more projected zygomatic and maxilla body with the increase in size. The processes of the zygomatic (i.e., frontal and temporal) and maxilla (i.e., frontal and zygomatic) become wider with age. For the Chubut sample (Figure 2b), the regression analysis indicates that 24.20% (p <.0001) of the total variation in shape is explained by changes in size. The facial skeleton becomes straight, with a marked projection associated with the increase in size. The zygomatic and maxilla are more anteriorly developed in larger individuals. The results of the linear model indicate that the allometric trajectories of both samples differ in their intercepts (p <.0001) but not in the slopes ($p = .074$), suggesting similar patterns of shape changes with size. Additionally the ANOVA shows that adults of both samples differ in size ($p = .00038$), indicating differences in the extension of the growth trajectories between samples.

The percentages of bone formation and bone resorption across age categories show a similar pattern in both samples, with a predominance of bone formation for the zygomatic bone whereas the maxilla exhibits a larger percentage of bone resorption (Figure 3). In Pampa Grande, the zygomatic bone (Figure 3a) shows a predominance of formation across ages, whereas the maxilla (Figure 3b) displays a progressive increase of resorption up to G3 (10.5–14.4 year-old) and a decrease of this activity in older age categories. Between G2 and G5 (4.5 years-old to young adults) the percentage of resorption is higher than formation. In Chubut, the bone formation is also the prevailing activity in the zygomatic bone (Figure 3c), although the activity of bone resorption is higher than in the Pampa Grande sample. The resorption activity decreases up to G3 and then increases toward the oldest age categories. The percentage of resorption also decreases and then remains almost similar in the maxilla (Figure 3d), with a predominance of bone resorption in G2 and G3 (4.5–14.4 years-old).

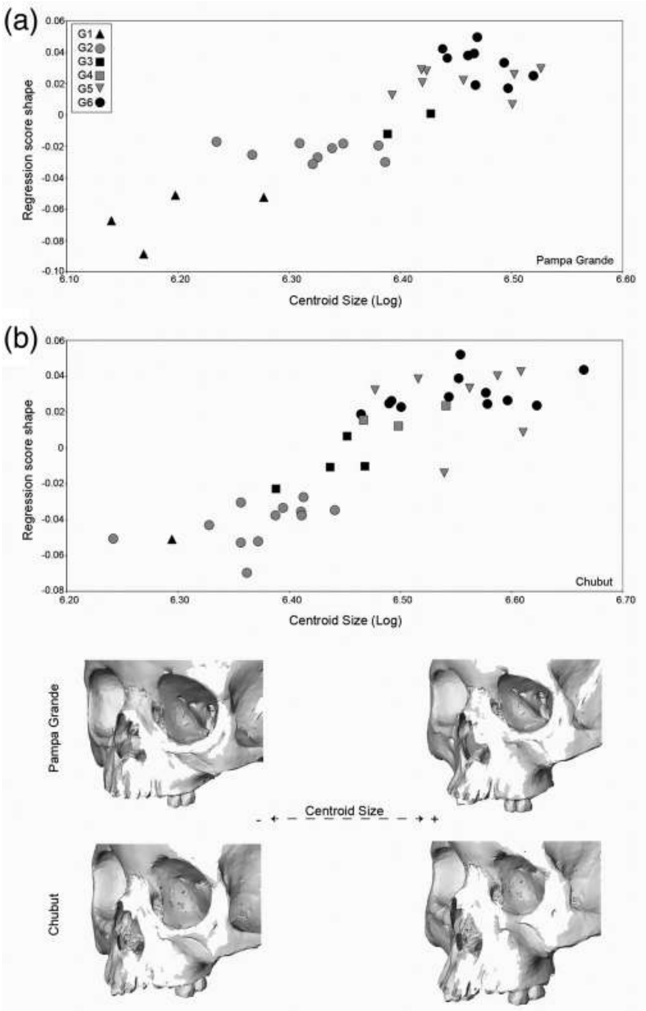

FIGURE 2 Regression of the shape variables on centroid size for the facial skeleton of Pampa Grande and Chubut samples. The morphings represent the variation in shape summarized in the extremes of size. Subadults: G1: <4.4 years; G2: 4.5–10.4 years; G3: 10.5–14.4 years. Adults: G4: 20–34 years; G5: 35–49 years

3.2 | Association between shape changes and bone remodeling patterns

The PLS analyses between facial shape and bone remodeling patterns in subadults and adults from Pampa

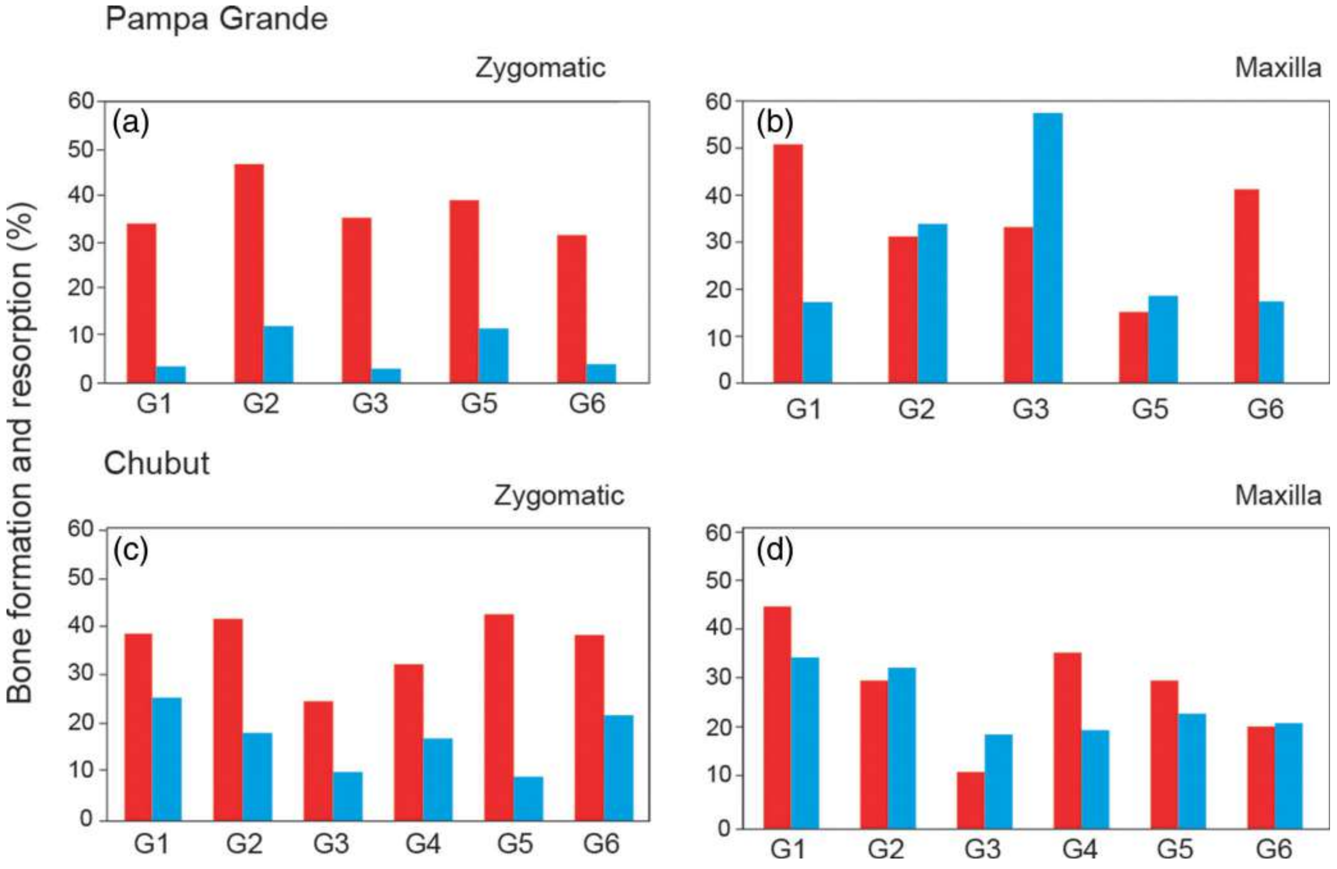

FIGURE 3 Percentage of bone formation and resorption for the zygomatic and maxilla in adults and subadults from Pampa Grande (a, b) and Chubut (c, d)

Grande and Chubut show a high association between both variables, although the *p*-values obtained were not significant (Figures 4–7). The results for the zygomatic bone of subadults from Pampa Grande show that individuals on the negative extreme of the first PLS axis have a higher proportion of formation and smaller areas of resorption, whereas those in the opposite extreme have a higher proportion of resorption on the anterior and posterior area (Figure 4a). Regarding shape variation, the individuals toward the negative scores of the first axis show a tall and wide zygomatic body, a long frontal process displaced to the medial side, a relatively large temporal process, and a small orbital margin. The first axis of bone remodeling of the adults from Pampa Grande (Figure 4b) separates the individuals with the highest proportion of formation toward the positive end and those with larger areas of resorption toward the negative values. The shape changes of the zygomatic bone show a higher and narrower frontal process and a longer temporal process along positive values compared to the morphologies toward the negative extreme.

The first PLS axis for the maxilla of the subadults of Pampa Grande separates individuals with a predominance of bone formation, placed toward the negative values, from individuals with a higher proportion of bone resorption, toward the positive extreme (Figure 5a). With respect to the changes in shape, the individuals placed in the negative values of the first PLS axis display a higher and wider frontal process in its upper portion, displaced toward the medial side, a longer but narrower zygomatic process, an alveolar process displaced upward, and a more rectangular maxillary body (longer and narrower) than the individuals placed in the positive values. The first block of bone remodeling for adult individuals (Figure 5b) separates those with the highest proportion of formation toward the positive end from those with more resorption in the negative side. Changes in shape are characterized by a wider frontal process at the base and narrower at the upper part, a wider zygomatic process, an alveolar process displaced upward and a maxilla body more laterally displaced.

Figure 6a shows the covariation between shape changes and bone remodeling patterns of zygomatic bone in the Chubut sample. The zygomatic bone of subadults changes from a predominance of resorption in the inferior border of the frontal process and in the inferior

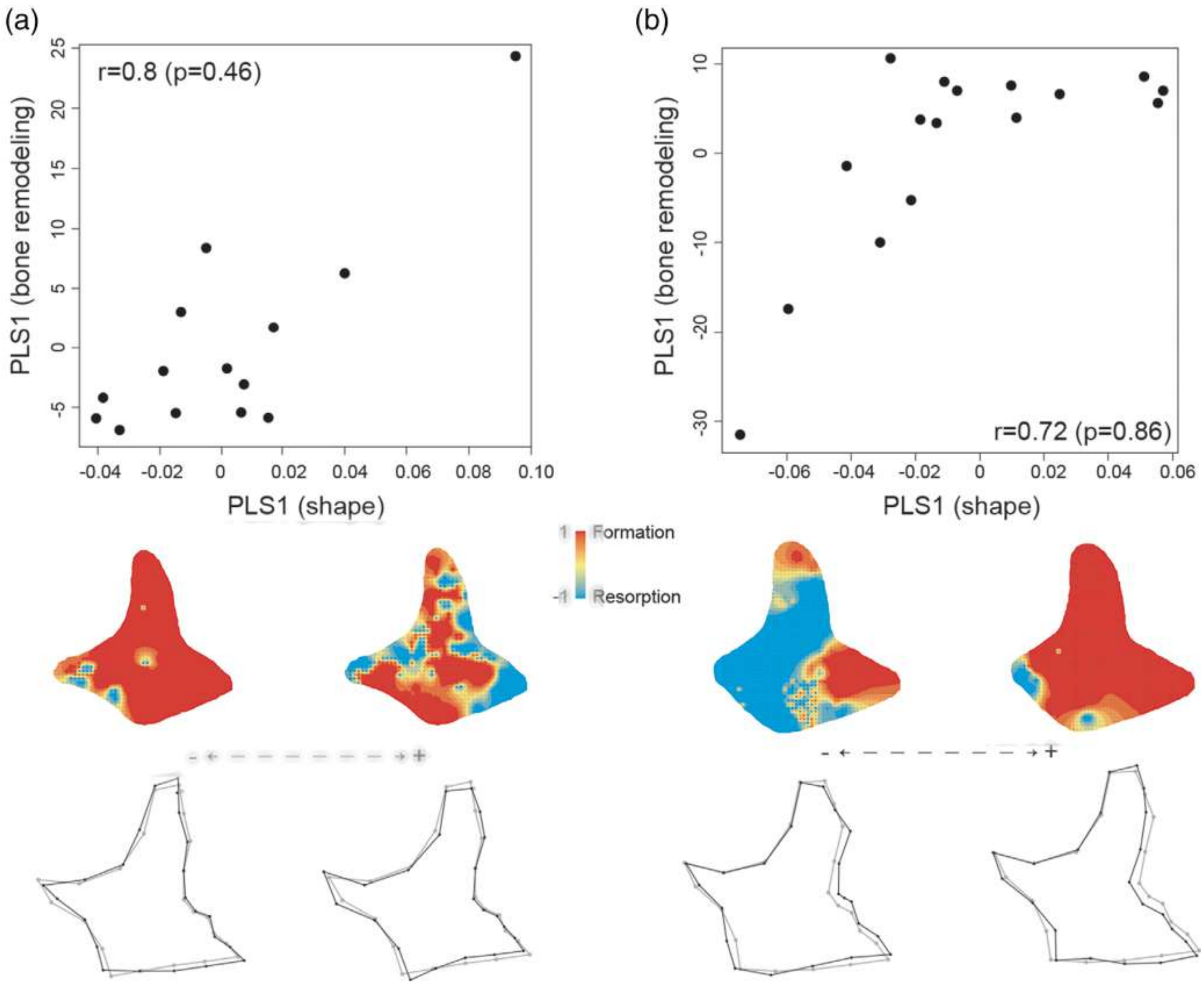

FIGURE 4 Partial least squares (PLS) analysis between shape and bone remodeling of the zygomatic in subadults (a) and adults (b) from Pampa Grande. To facilitate the visualization of shape changes, only the points on the contour are represented in the wireframe. The gray lines represent the consensus and the black lines represent the shapes at the extremes of the first PLS axis. The bone remodeling maps correspond to the extreme individuals along the first PLS axis

orbital margin to a predominance of formation from negative to positive values of the first PLS axis (Figure 6a). The changes in shape associated with bone remodeling show that individuals at the negative extreme of the first PLS axis have a shorter and wider frontal process, a longer zygomatic body posteriorly displaced, and a wider temporal process than those toward the positive values. The PLS analysis for adults shows that those individuals with higher proportions of formation are on the negative values (Figure 6b). This was associated with shape changes characterized by a frontal process medially displaced, a longer and narrower body, and a relatively larger temporal process.

The first PLS axis of the maxilla of subadults from Chubut separates bones with a predominance of formation in the positive extreme from those with a higher proportion of resorption toward the negative values (Figure 7a). With regard to shape variation, the specimens toward the positive values of the first PLS axis have a smaller and wider frontal process in the upper part, a relatively taller alveolar process, and a medial displaced maxillary body. The PLS analysis for adults shows that the first axis of bone remodeling separates the individuals with higher proportion of formation in the negative extreme and those with more resorption toward the positive values (Figure 7b). The first axis that summarizes the covariation in shape, shows that the morphologies in the negative values present a wider and shorter frontal process, an inferiorly displaced alveolar process, and a more rectangular maxillary body.

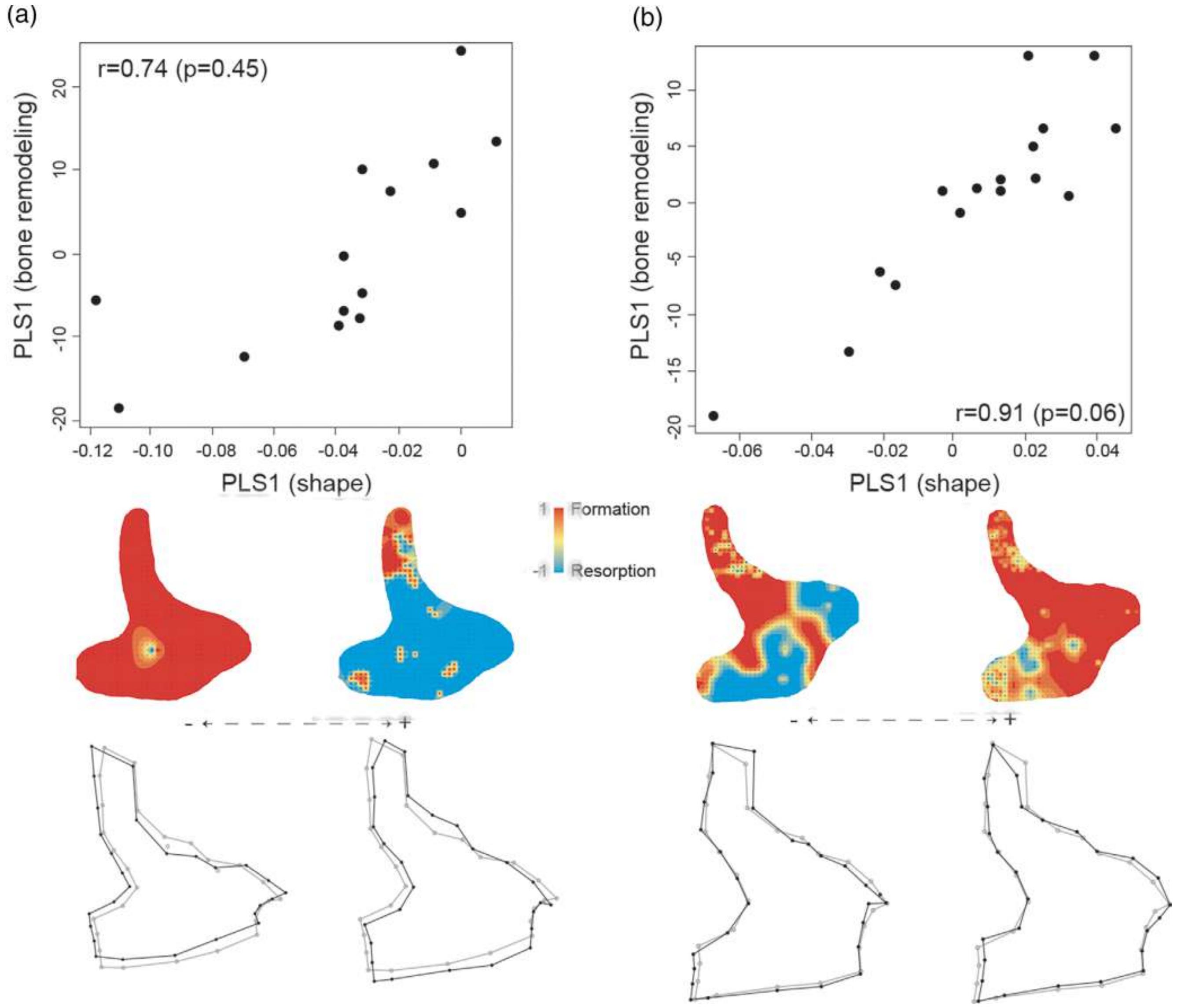

FIGURE 5 Partial least squares (PLS) analysis between shape and bone remodeling of the maxilla in subadults (a) and adults (b) from Pampa Grande. To facilitate the visualization of shape changes, only the points on the contour are represented in the wireframe. The gray lines represent the consensus and the black lines represent the shapes at the extremes of the first PLS axis. The bone remodeling maps correspond to the extreme individuals along the first PLS axis

4 | DISCUSSION AND CONCLUSIONS

This study assessed the covariation between changes in facial shape with the underlying bone cell activity throughout the ontogenetic trajectory in two Amerindian populations. The complete remodeling maps obtained allowed us to apply multivariate statistical analyses to compare the bone remodeling patterns within and between samples. The quantification of the bone remodeling activity also allowed the integration of data from different levels of phenotypic variation (e.g., histological and morphological) using multivariate statistics. Our findings indicate that the changes in the patterns of bone remodeling are associated with morphological variation of the middle face.

We found similar shape changes along the ontogenetic trajectory in the two samples analyzed here—Pampa Grande and Chubut. Overall, the increase in size in the maxilla and the zygomatic was associated with an anterior projection and a straight body shape in both bones. Adult individuals displayed an enlargement of the frontal and temporal processes of the zygomatic bone as well as the frontal and zygomatic processes of the maxilla, which was more pronounced in the Chubut sample. The similarity of shape changes in both samples suggests that the morphological disparity among populations arises early in the ontogeny, as it has been previously

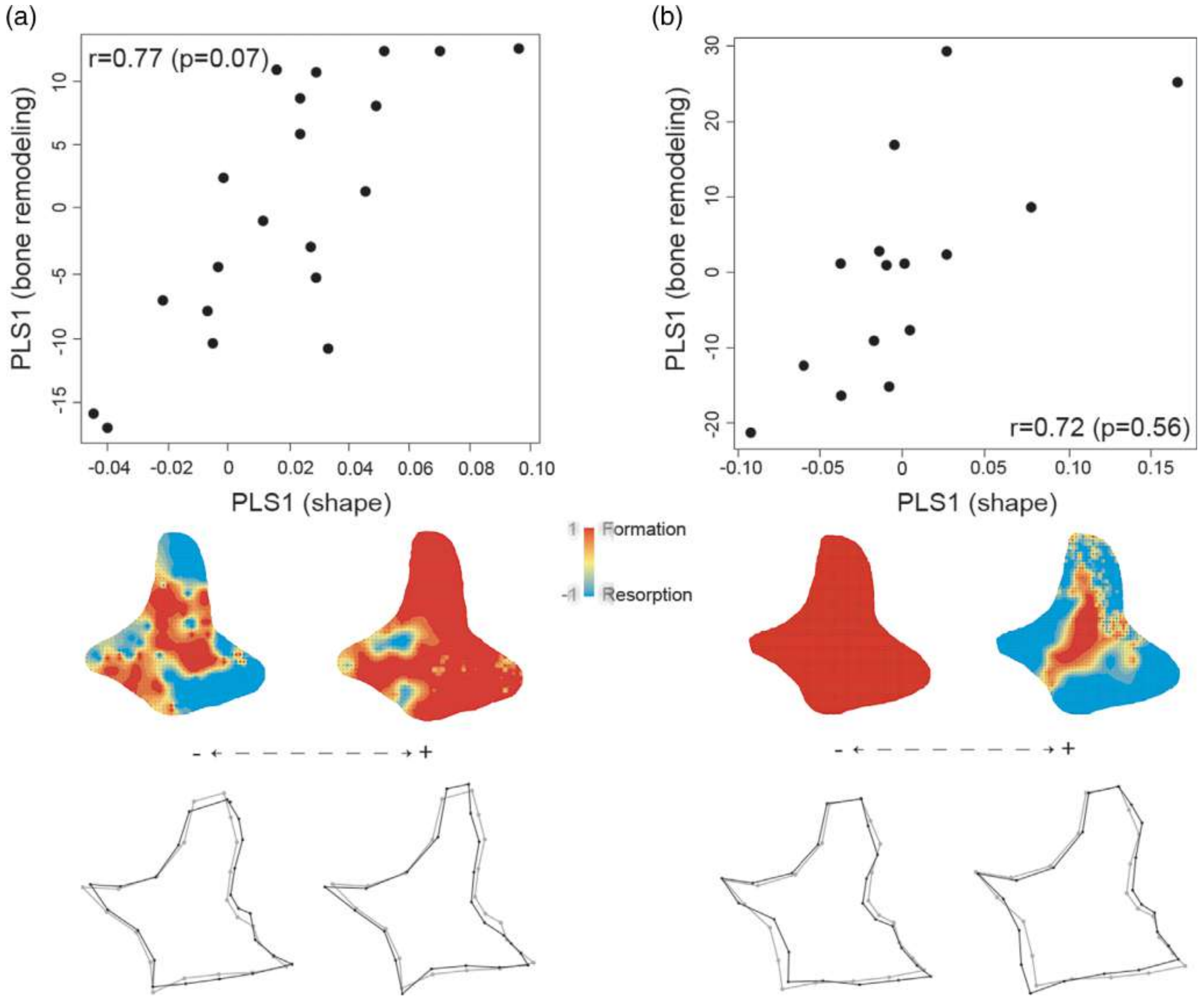

FIGURE 6 Partial least squares (PLS) analysis between shape and bone remodeling of the zygomatic in subadults (a) and adults (b) from Chubut. To facilitate the visualization of shape changes, only the points on the contour are represented in the wireframe. The gray lines represent the consensus and the black lines represent the shapes at the extremes of the first PLS axis. The bone remodeling maps correspond to the extreme individuals along the first PLS axis

discussed (Barbeito-Andrés et al., 2011; Eyquem, Kuzminsky, Aguilera, Astudillo, & Toro-Ibacache, 2019; Gonzalez et al., 2010, 2011). In this sense, shape differences across South American populations were found to be accentuated by changes associated with the increase in size (Barbeito-Andrés et al., 2011; Eyquem et al., 2019; Gonzalez et al., 2010). Given the significant differences in size between adults from Pampa Grande and Chubut, the extension of the growth trajectories seems to be a relevant factor contributing to craniofacial differentiation in the two samples analyzed here. As previous studies have shown, individuals younger than 5 years-old have a similar skull size in both samples, followed by an increase in the morphological differentiation resulting from the larger size attained by the individuals from Chubut (Barbeito-Andrés et al., 2011; Gonzalez et al., 2011).

The analysis of bone remodeling also showed a similar trend in the amount of formation and resorption activities across ages for the zygomatic and the maxilla in the two samples analyzed, although the Chubut sample showed a higher proportion of resorption activity. Even though bone formation was predominant, a larger proportion of bone resorption was observed for the maxilla than for the zygomatic. Furthermore, both samples had a predominance of resorption in the maxilla from 4.5 to 14.4 years-old. These results agree with previous studies of bone remodeling of the middle face in ontogenetic samples of modern populations from different geographic

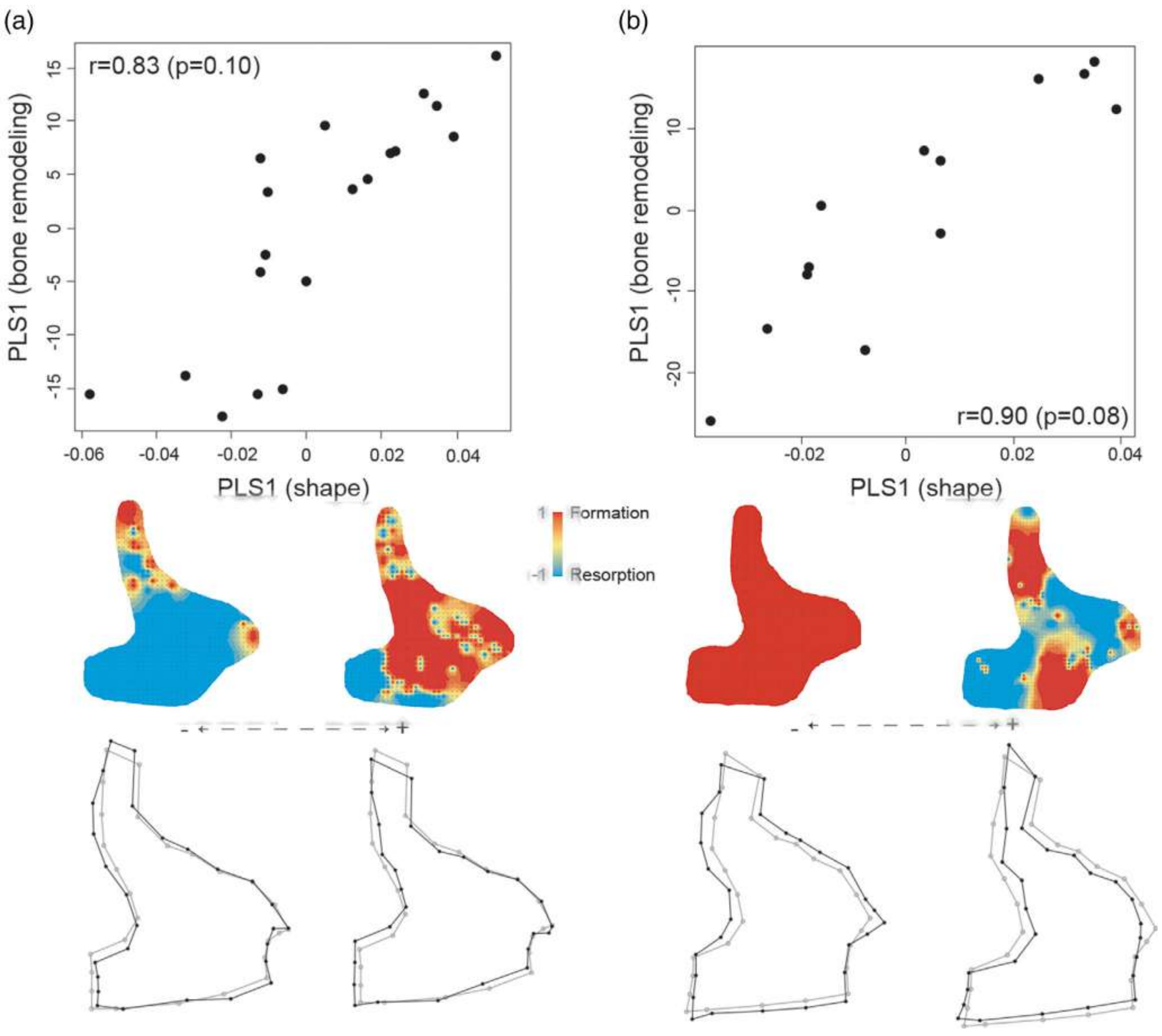

FIGURE 7 Partial least squares (PLS) analysis between shape and bone remodeling of the maxilla in subadults (a) and adults (b) from Chubut. To facilitate the visualization of shape changes, only the points on the contour are represented in the wireframe. The gray lines represent the consensus and the black lines represent the shapes at the extremes of the first PLS axis. The bone remodeling maps correspond to the extreme individuals along the first PLS axis

origin, suggesting that there is a common sequence of bone remodeling changes associated with facial growth in human populations (Enlow & Bang, 1965; McCollum, 2008; Schuh et al., 2020).

However, there is a large variability across ages and populations in the distribution of the areas of bone formation and resorption. For the zygomatic, the presence of resorption in the anterior region and in the internal surface of the bone, and formation in the posterior region and in the external surface, has been considered to contribute to the posterior positioning and verticalization of the bone, as well as to its lateral growth (Enlow & Bang, 1965; Enlow & Hans, 1996; Martinez-Maza et al., 2013). This pattern is quite consistent for individuals from Pampa Grande, but not for individuals from Chubut, where large areas of resorption were observed on the area of insertion of the masseter (Brachetta-Aporta et al., 2019a). The presence of resorption across the inferior border of the zygomatic has also been found in adults from a modern sample (Martinez-Maza et al., 2013).

The variation in bone remodeling is higher for the maxilla, principally in two areas: the alveolar process and the canine fossa-zygomatic process. Conversely, the frontal process has a very consistent pattern of bone activity across populations, characterized by the presence of deposition (Enlow & Bang, 1965; Enlow & Hans, 1996; Kurihara et al., 1980). In general, the anterior maxilla has a prevalence of resorption at early ages with an increase in deposition among adults (Brachetta-Aporta et al., 2019a, 2019b; Enlow & Bang, 1965; Kurihara et al., 1980; Martinez-Maza et al., 2013; Schuh et al., 2020). We found that in the Chubut sample, the extension of resorption across the alveolar process persists into adulthood, which could be related to the high load demands among hunter-gatherers (Brachetta-Aporta et al., 2019a). However, the same has not been found for the Inuits, a population also exposed to high masticatory loads (Schuh et al., 2020). Such discrepancies might be explained by differences in the facial morphology between populations. Since the alveolar processes correspond with tension strains which in turn support the presence of deposition (Brachetta-Aporta & Toro-Ibacache, 2021), the Inuit skull morphology may better withstand masticatory forces. Additionally, given the small size of the Inuit sample and some differences between studies in the parameters used for the spatial analysis, we cannot discard the effect of methodological aspects on the discrepancies in the levels of variation in the bone remodeling patterns across populations. These results underline the need to incorporate more populations and follow consistent and standardized methods for the spatial analysis of bone remodeling changes.

The integrated analysis of bone morphology and remodeling showed a strong, although non significant, association between morphological variation and the distribution of areas of bone formation and resorption. A longer zygomatic was associated with the presence of formation in the body and the frontal process. Bone resorption in the anterior area, which is considered to contribute to the posterior retraction of the bone (Enlow & Bang, 1965; Enlow & Hans, 1996), was associated with a larger and more laterally projected zygomatic in adults. We also found a verticalization of the face associated with the presence of formation in the posterior area of the zygomatic for Pampa Grande and adults from Chubut, contrasting with the proposed relation between the presence of resorption on the posterior area of the zygomatic and the verticalization of the face that occur with age (Enlow & Bang, 1965: Enlow & Hans, 1996; Martinez-Maza et al., 2013).

Regarding the maxilla, the presence of larger areas of bone formation was associated with a wider frontal process in both samples, and with a decrease of height in individuals from Chubut. Similarly, an extension of formation areas was associated with an upper displacement of the alveolar process in individuals from Pampa Grande and the subadults from Chubut, as well as an increase of height of the alveolar process in adults from Chubut. An inferior displacement has been previously associated to the presence of anterior resorption (Brachetta-Aporta et al., 2019b; Enlow & Bang, 1965; Martinez-Maza et al., 2013; Schuh et al., 2020), while the increase in height and width of the maxilla correspond to bone deposition (Brachetta-Aporta et al., 2019b; Schuh et al., 2019). However, we found a greater anterior projection associated with bone resorption in individuals from the Chubut sample. Such inconsistencies may indicate that differences in the rate of formation and/or resorption rather than in the spatial extension of cell activity are associated with facial projection in this population. One of the limitations for studying remodeling rates is that they cannot be inferred from bone surface but from histological sections, which is an invasive method difficult to apply in prehistoric samples (García Gil et al., 2016). Another alternative previously discussed is a differential contribution of the bone remodeling of maxillary tuberosity to the displacements (Brachetta-Aporta et al., 2019a). Even though the horizontal elongation of the dental arch is attributed to a continuous deposition of bone on the tuberosity (Enlow & Bang, 1965; Enlow & Hans, 1996), the remodeling pattern of this structure seems to be more variable than previously proposed (Martinez-Maza et al., 2013). On the other hand, the bone remodeling and fusion of the pterygoid plate with the maxillary tuberosity could also contribute to differences in facial projection between samples (Bromage & Boyde, 2008).

Overall, our findings provide clues about the processes and mechanisms of bone development involved in the facial morphological differentiation between the populations under study. The differences found in the bone remodeling maps indicate that the distribution of bone formation and resorption contributed to the morphological differentiation in the middle face between the two samples analyzed here. The main differences were observed in areas related to mechanical loadings, with the sample of hunter-gatherers showing a greater extension of resorption surfaces, extended even to adult ages. This persistence of the bone resorption activity in adults has been proposed as a consequence of mechanical strains due masticatory forces (Brachetta-Aporta et al., 2019a; Martinez-Maza et al., 2013), which particularly for Chubut sample is also supported by a greater facial robustness (Bernal et al., 2006; González-José et al., 2005; Sardi et al., 2005). Particularly, changes in the periosteal functional matrix, including the masticatory muscles, have a direct effect on the skeletal units producing modifications in the distribution of areas of bone formation and resorption as well as in the rate of remodeling (i.e., including the onset, offset, and rate of activity; Carlson, 2005; Enlow & Hans, 1996; Martin et al., 2015; Moss, 1997; Moss & Salentijn, 1969). Other functional matrices that influence the development of the zygomatic and the maxillary bones also need to be taken into account (Cheverud, 1982; Moss & Salentijn, 1969). These bones constitute the floor and the lateral wall of the orbit respectively, so they are related to the development of the orbital soft tissues. Likewise, the maxilla is associated with the airways and the development of the paranasal sinuses at the middle level, and with the teeth at the level of the hard palate and the alveolar arch (Cheverud, 1982; Lieberman, 2011). Altogether, the development and displacements of capsular and periosteal functional matrices affect bone morphology until adulthood (Lieberman, 2011; Moss & Salentijn, 1969; Moss & Young, 1960; Sardi & Ramírez Rozzi, 2005). Despite this, a previous analysis of the association between the maxillary and zygomatic bone remodeling and the size of the maxillary sinus and eyeball cavities of subadults and adults from Pampa Grande and Chubut showed absence of co-variation between them (Brachetta-Aporta, 2018). However, this question needs further studies. Until now, the remodeling patterns on bone surface have been evaluated in association to bone shape, while the generation of comparative models that test the osteogenic activity in response to factors contributing to population differentiation (e.g., muscular loading) is just being approached (Martínez-Vargas et al., 2017; Walters, Crew, & Fyfe, 2019; Brachetta-Aporta & Toro-Ibacache, 2021). This will allow us to discuss in greater depth the factors that model the craniofacial morphological variation at intra- and inter-population level.

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AUTHOR CONTRIBUTIONS

Natalia Brachetta Aporta: Conceptualization, Data curation, Formal Analysis, Investigation, Methodology, Visualization, Writing – Original draft, Writing – review & editing. **Paula Gonzalez:** Conceptualization; funding acquisition; investigation; methodology; resources; supervision; writing-review & editing. **Valeria Bernal**: Conceptualization, Funding acquisition, Investigation, Methodology, Project administration, Resources, Supervision, Writing – review & editing.

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Additional supporting information may be found online in the Supporting Information section at the end of this article.

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