Bone Modeling Patterns and Morphometric Craniofacial Variation in Individuals from Two Prehistoric Human Populations from Argentina

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ABSTRACT

Native human populations from South America display high levels of craniofacial variation encompassing gracile and robust skulls. Nevertheless, the processes of bone modeling by which morphological variation among populations were attained, remain poorly understood. Here we analyze the relationship between patterns of bone formation and resorption and morphometric variation in the upper face of adults belonging to farmers and hunter-gatherers from northwestern and south Argentina. Our analyses reveal a common pattern of bone modeling of the malar bone characterized by the presence of formation areas. Thus, the larger size and greater development of malar bone exhibited by hunter-gatherers would be linked to a greater magnitude of bone formation activity. Conversely, the glabella and the superciliary arch presented both formation and resorption areas with a variable distribution among individuals. In the extreme corresponding to more robust morphologies, the great development of the glabella is related to the presence of large formation fields, both in the upper region and toward the frontonasal suture. The less robust morphologies show resorption fields at the upper margin of the glabella, which would contribute to the weaker development of this region. The superciliary arch showed a complex relationship between its morphometric and histological variation; the individuals located at both extremes of the shape space presented large resorption areas located on its upper margin. Overall, our results show the existence of intraspecific variation in the patterns of bone modeling in the human upper face. Anat Rec, 297:1829–1838, 2014. © 2014 Wiley Periodicals, Inc.

Key words: bone formation and resorption; geometric morphometrics; human face

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Native human populations from southern South America are characterized by their high level of craniofacial morphological variation. Several works have shown the existence of a pattern of variation in adult individuals whose extremes are occupied by small skulls with gracile facial structures—glabella, supraorbital arch, and zygo-maxillary region—corresponding to individuals from farming groups, and larger skulls with more robust facial features corresponding to hunter-gatherer groups (González José et al., 2005; Sardi et al., 2005; Pucciarelli et al., 2006; Perez and Monteiro, 2009; Perez et al., 2011). Moreover, recent analyses suggest that the pattern of interpopulation variation in shape and size is evident at the age of 5 years, although it becomes more pronounced among adults (González et al., 2010, 2011; Barbeito Andrés et al., 2011). So far, these studies focused at the macroanatomical scale, and thus they allowed only a partial and indirect approach to the study of the processes that act at the cellular level and which are essential to understand the mechanisms underlying the craniofacial morphology of adult individuals (e.g. Enlow, 1963; Kurihara et al., 1980; Bromage, 1989; Enlow and Hans, 1996; McCollum, 2008; Lieberman, 2011; Lacruz et al., 2013; Martinez-Maza et al., 2013).

According to the Enlow's counterpart principle (Enlow et al., 1969; Enlow and Hans, 1996) and the functional matrices theory (Moss and Young, 1960; Moss and Rankow, 1968; Moss and Salentijn, 1969; Moss, 1997a,b), the skull grows through interrelated complex processes involving the growth by bone modeling mechanism and displacements of its skeletal elements to maintain a functional and structural balance (e.g. Moss and Young, 1960; Moss and Salentijn, 1969; Enlow and Hans, 1996; McCollum, 2008; Lieberman, 2011). The bone modeling mechanism (a process also termed as remodeling; Enlow and Hans, 1996; see discussion in Martinez-Maza et al., 2006) consists in the coordinated and uncoupled activity of two cellular groups, osteoblasts (bone forming cells) and osteoclasts (bone resorbing cells). During development, bone growth is influenced by many factors including different genetic, biomechanical and hormonal factors (Enlow and Hans, 1996; O'Higgins et al., 1991) as well as by the growth of the functional spaces (cranial, orbital, nasal, and oral cavities) and the soft tissues in which they are embedded (e.g., brain, muscles, connective tissues) (Moss and Young, 1960; Enlow and Hans, 1996; see also Lieberman, 2011 and cites there in). Consequently, craniofacial bones change their size and shape as well as their relative position within the craniofacial system maintaining the proper bone alignment, function and proportionate growth (by means of drift, displacement, and rotation; Moss and Young, 1960; Björk, 1969; Björk and Skieller, 1972, 1976; Enlow and Hans, 1996; see also a review in Martínez-Maza et al., 2006). These factors ultimately regulate the onset, offset and rate of activity as well as the spatial distribution of the areas of bone formation and resorption (Enlow and Hans, 1996; Martin, 2000; Robling et al., 2006). Changes in any of these parameters will contribute to the morphological differences observed among species and populations (Lieberman, 2011).

One of the approaches to study the dynamics of bone modeling that underlie morphological variation, is based on the identification of microstructural features generated by the cellular activities of tissue formation and resorption on the surface of bone (Enlow, 1963; Boyde, 1972; Bromage, 1989; Enlow and Hans, 1996; Martinez-Maza et al., 2010). Such data are used to build maps of bone modeling that show the distribution of areas of cellular activity, whose interpretation in the field of craneofacial biology provides insight regarding the directions of growth in the various bone regions (Enlow and Hans, 1996). The development of a specific nondestructive methodology for these types of studies has allowed the analysis of the craniofacial complex of fossil and living primates, and the particular pattern of each species has been established (e.g., Bromage, 1989; O'Higgins et al., 1991; McCollum, 1999, 2008; Rosas and Martinez-Maza, 2010; see also a review of these works in Martinez-Maza et al., 2006; Martinez-Maza et al., 2011, 2013). It has also been suggested that some differences among human populations exist, although the available data come exclusively from a reduced number of recent populations of European origin, and thus the range of variation of the species remains poorly understood (Kurihara et al., 1980; Hans et al., 1995; McCollum, 2008; Martinez-Maza et al., 2013).

The main goal of this work is to explore the relationship between patterns of bone modeling in periosteal surfaces and morphometric variation, in the upper region of the face of adult individuals belonging to populations from northwestern and south Argentina, which represent the extremes of morphological variation described for South America. In particular, the individuals that show greatest differentiation in the morphometric analyses are also expected to exhibit the greatest differences regarding the distribution of fields of bone modeling. To describe the axes of greatest variation of the shape and size of craniofacial structures, we used multivariate statistical analyses derived from geometric morphometrics. The microstructure of the bone surface was studied using high resolution bone replicas that were analyzed under incident-light microscope. Bone modeling maps made from these data were compared with the pattern of morphometric variation at macroanatomical level.

MATERIAL AND METHODS

Osteological Samples

We analyzed adult individuals from archaeological sites located in the lower valley of the Chubut river (Chubut province, Argentine Patagonia) and Pampa Grande (Salta province, Northwestern Argentina). These materials are deposited in the Anthropology Division of the Facultad de Ciencias Naturales y Museo, Universidad Nacional de La Plata (Buenos Aires, Argentina).

The sample from Chubut (Ch) consists of 34 individuals from burial sites located in the vicinity of the city of Trelew, in Chubut province (Lehman-Nitsche, 1910). This sample corresponds to a population of hunter-gatherers, radiocarbon dates assign it a late Holocene age, between about 900 and 1600 years BP (Béguelin, 2009). The sample from Pampa Grande (PG) includes 21 individuals from burial sites in the hill system of Las Pirguas (Departament of Guachipas, Salta province) and corresponds to farming groups. The associated cultural materials were assigned to the so-called Candelaria culture, whose chronology has been estimated between

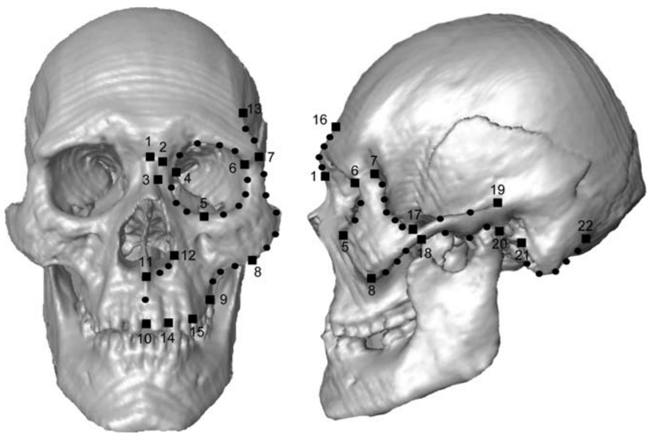

Fig. 1. Landmarks (squares) and semilandmarks (circles) registered on the skull. Facial view: 1: nasion, 2: nasofrontal; 3: nasomalar; 4: dacrion, 5: zygoorbital; 6: anterior frontomalar; 7: temporal frontomalar; 8: anterior zygomaxillary; 9: ectomolar; 10: prostion; 11: subspinal; 12: alar; 13: frontotemporal; 14: alveolar margin between M1 and M2 *; 15: alveolar margin between C and PM1*; Howells, 1973; Buikstra and Ubelaker, 1994; *this work. Lateral view: 16: posglabellar; 17–22: Reproduced from Gonzalez et al., 2010.

1500 and 1400 years BP (González, 1972; Baldini et al., 2003).

Estimations of age and sex of the individuals were made on the basis of morphological indicators from craneofacial structures. Sex was estimated using the degree of development of the glabella, supraorbital margin, mastoid process, supramastoid crest, and nuchal crest (Buikstra and Ubelaker, 1994; Walrath et al., 2004). Estimations of age of death were based on eruption of the third molar and degree of obliteration of the spheno-basilar suture and of the ectocranial sutures of the latero-anterior region (Meindl and Lovejoy, 1985). On the basis of these indicators, the individuals were assigned to one of the following age categories: young adult (25- to 35-years-old), middle-aged adult (35- to 45-years-old), and old adult (45- to 60-years-old) (Buikstra and Ubelaker, 1994).

Morphometric Analysis

The morphological variation of craniofacial traits was quantified by means of geometric morphometric techniques. Digital images of the skulls in lateral and frontal views were obtained using an Olympus SP 350 digital camera. The skulls were positioned on the Frankfurt plane. The lateral view images were taken at a 30 cm-distance from the euryon and those of the frontal view, at 25 cm away from the prostion. In this work we analyzed the upper region of the face, which includes the superciliary region, the glabella and the malar region. The coordinates of 12 landmarks and 25 semilandmarks were digitized on the lateral view, and 15 landmarks and 24 semilandmarks on the frontal view (Fig. 1) (Gonzalez et al., 2010). The point coordinates were registered using the software tpsDIG 1.40 (Rohlf, 2008).

The differences between configurations of landmarks and semilandmarks due to position, orientation, and scale of individuals were removed by means of a Generalized Procrustes Analysis (Rohlf and Slice, 1990). The semilandmarks were slid along the outlines using the minimum Procrustes distance criterion (Bookstein et al., 2002; Perez et al., 2006). This procedure resulted in a set of Procrustes coordinates that describe the shape differences among the individuals analyzed. Centroid size (CS; square root of the sum of squared distances between each landmark and semilandmark to the configuration centroid) was used as a measure of size of the facial skeleton (Bookstein, 1991).

Finally, in order to describe the axes of greater variation of the craniofacial structures, we calculated *Relative warps* (RW) using the Procrustes coordinates. This analysis consists of obtaining the eigenvalues and eigenvectors of the covariation matrix of Procrustes coordinates by singular value decomposition analysis. This transformation results in new variables, the principal components, which represent different percentages of the total shape variation. Because each successive component represents a greater amount of the total shape variation, only a few axes (usually two) are necessary to represent the shape variation among individuals. This analysis was made using the software program tpsRelw 1.44 (Rohlf, 2008). The first RW was plotted against the log-transformed centroid size (logSC) to describe differences among individuals in the shape-size space.

Histological Analysis

The best preserved individuals from both samples were selected for histological study. A macroscopic analysis was used to determine the state of preservation of bony surfaces; individuals with taphonomic alterations, malformations, pathologies, alveolar resorption due to ante-mortem tooth loss, trauma or abscesses, among others, were excluded from this analysis because these factors may hinder the identification of fields of bone formation/resorption and modify the normal pattern of bone modeling. Likewise, the sample selected for the histological analysis includes only individuals that represent the extremes of morphological variation according to the data obtained from the geometric morphometric analyses. Thus, the selected sample consists of 10 individuals, five from Pampa Grande and five from Chubut (Table 1).

The identification of bone formation and resorption fields requires microscopic analysis of the bone surface. This inspection was performed using a nondestructive method that consists of obtaining high-resolution casts of the bone surface, which were then observed under incident-light microscope (Martinez-Maza et al., 2010). Before making the casts, the surfaces of anatomical regions were cleaned by applying 60% alcohol using a brush with soft and fine bristles to avoid leaving marks. Subsequently, negative casts of the upper face (glabella, malar region, and supraorbital arch) were made by applying low-viscosity silicon (Coltène® President light body) on the bone surface. This cast was made to produce the positive molds using epoxy resin (Tolken®), which were covered by a fine layer of gold (~150–200 Å) to give electric conductivity to the samples. This procedure was done at the Servicio de Microscopía Electrónica de Barrido y Microanálisis (CINDECA) using a Balzers metalizer. To facilitate the observation and recording of microstructural bone features, a 5 × 5 cm^2 grid was drawn on the positive molds.

TABLE 1. Individuals from Chubut and Pampa Grande included in the histological analysis

			Superciliary Arch		Glabella		Malar	
Individual	Sex	Age group	B. For	B. Res	B. For	B. Res	B. For	B. Res
PG17706	Female	YA	X	X			X	
PG17716	Female	YA					X	
PG17726	Female	MA		X			X	
PG17749	Male	YA	X		X	X	X	
PG17690	Male	MA				X	X	
Ch1081	Female	OA		X		X		
Ch1100	Female	MA	X	X				
Ch1112	Female	MA					X	
Ch1136	Female	YA		X	X		X	

Age group codes: Young Adult (YA), Middle-age Adult (MA), and Old-Adult (OA); Cellular activity codes: bone formation (B. For) and bone resorption (B. Res). Blank spaces correspond to areas for which no histological information was recorded during the examination of samples.

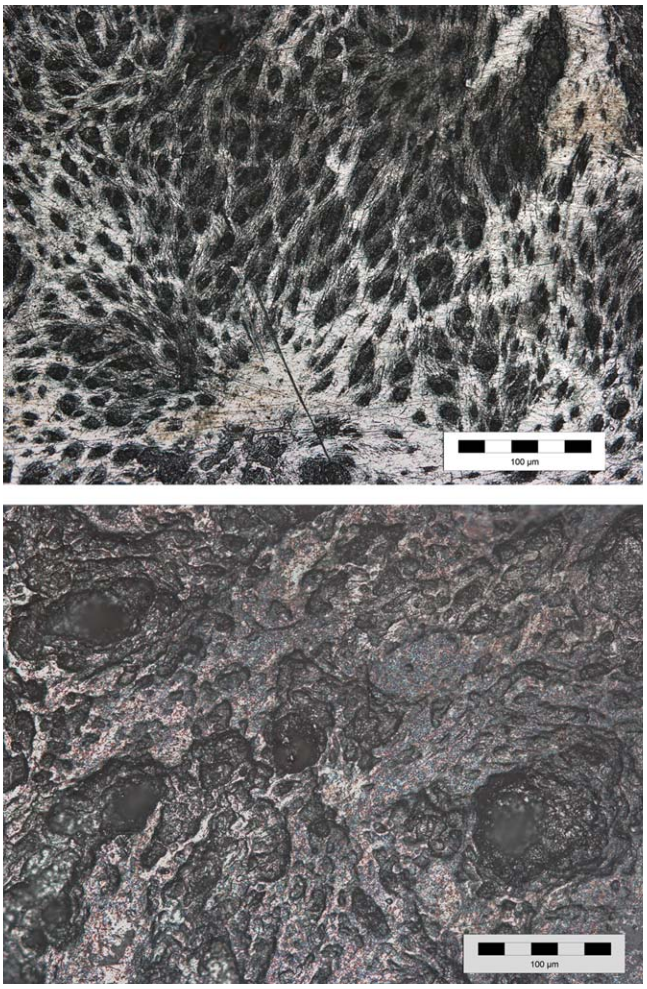

Fig. 2. Comparison of bone formation (superior) and bone resorption surfaces (inferior) from the glabella of the specimen PG 17749 2R. Images obtained with an incident-light optical microscope (20×).

Replicas of bone surfaces were observed under an incident light optical microscope (ILM) with a 20× NA 0.40 objective. Martinez-Maza et al. (2010) demonstrated the validity of the ILM to identify the histological features related to bone modeling activities as an alternative method to that proposed by Bromage (1984, 1989) using scanning electron microscopy (SEM). Identification of bone formation and resorption activity was carried out following the descriptions by Bromage (1984) and Martinez-Maza et al. (2010) (Boyde, 1972). In brief, bone formation surfaces are characterized by the presence of packs of collagen fibers generated by osteoblasts, which are visible as parallel elongated bundles arranged in a predominant direction (Fig. 2). Resorption surfaces characteristically present randomly distributed concavities of variable size and shape known as Howship's lacunae, produced by osteoclast activity (Fig. 2). Digital images of representative surfaces were obtained using an Olympus BX50 microscope (objective 20× NA 0.50) fitted with an Olympus DP71 camera using the software program cellSens Dimension v1.7 (Olympus).

Previous works analyzing anorganic bone with a SEM indicated that in addition to the type of cellular activity, it is also possible to establish the state of bone modeling activity by identifying the active or inactive fields of bone formation and resorption (Jones and Boyde, 1970; Boyde, 1972; Bromage, 1984, 1989; Marks et al., 1996). The characteristics outlined above would correspond to active fields, whereas inactive bone formation surfaces show anastomosed and less defined collagen fibers, so that the surface has a smooth, shiny appearance. This type of surfaces result from the cessation of osteoblast activity; when this happens, mineralization front advances, first involving the collagen fibers (observed in active surfaces) and then affecting the fundamental matrix of the zone, thus originating the smoother surface (Jones and Boyde, 1970; Boyde, 1972; Marks et al., 1996). Inactive bone resorption areas would present shallower Howship's lacunae and the concavities would have a less defined margin (Jones and Boyde, 1970; Boyde, 1972). The identification of inactive bone modeling fields in fossil remains becomes difficult because of alterations of the bone surface caused by taphonomic processes, including manipulation during laboratory analyses (Bromage, 1984, 1989). Although identification of the state of cellular activity would provide valuable information to infer growth dynamics, the difficulty of differentiating active from inactive fields could introduce errors in the interpretation of the growth model. Specimens analyzed in this study present altered surfaces, and thus we

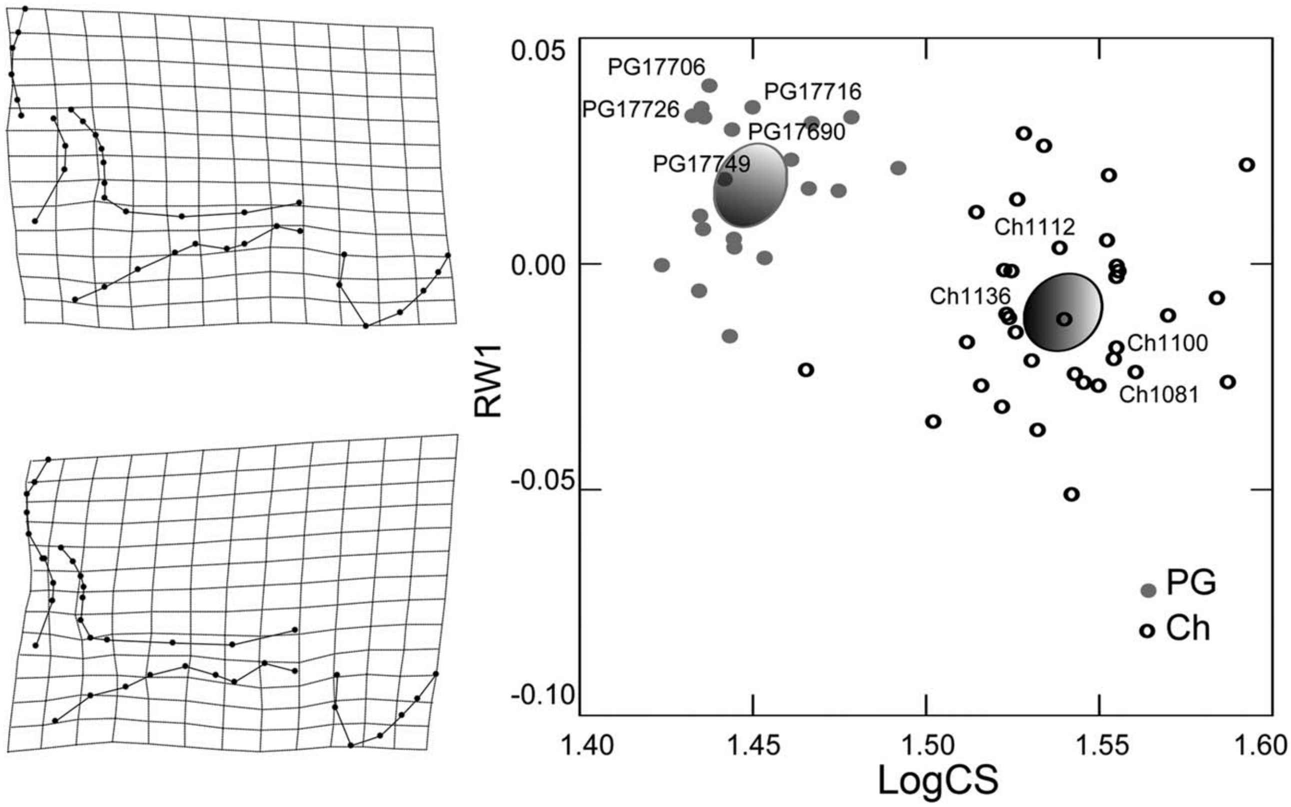

Fig. 3. Scatterplot of Chubut (Ch) and Pampa Grande (PG) individuals in the form space represented by Relative Warp 1 (RW1) and the log-transformed centroid size (Log CS) of the facial skeleton in lateral view. The deformation grids illustrate the shape changes at the negative and positive extremes of the first axis (RW1).

have identified fields of bone formation and resorption without assigning cellular activity states.

By recording these histological data, we were able to establish the pattern or map of bone modeling for each individual, which shows the distribution of bone formation and resorption activity.

RESULTS

Patterns of Morphometric Variation

The results of the Relative Warps analysis of the coordinates in lateral view indicate that the two samples differ along the first axis, which explains 23.84% of the total shape variation (Fig. 3). The Chubut individuals are distributed toward extreme negative values, while PG individuals occupy the opposite end. The deformation grids indicate that Chubut individuals are characterized by greater development of the glabella (Fig. 3). Likewise, the two samples show marked differentiation regarding the size of facial structures (Fig. 3). According to these results, the samples from Pampa Grande and Chubut are significantly differentiated in the form space.

Similar results are obtained from the analysis of the face in frontal view, which show marked differentiation of the samples along RW1, which accounts for 29.32% of the variation (Fig. 4). The deformation grids indicate that the individuals located on the negative extreme of RW1 are characterized by greater relative facial height and smaller orbit than those located on the positive end, which correspond to the Pampa Grande sample (Fig. 4). Similarly, the morphologies observed toward positive RW1 values show greater lateral projection of the malar bone. The individuals from the Chubut sample tend to be larger than those from Pampa Grande, but unlike the case of the lateral view, the structures in frontal view exhibit greater overlap in size.

Patterns of Bone Modeling

On the basis of the arrangement of individuals in the form space (RW1 + logCS) and taking into account the degree of preservation of the bone surface, individuals representing the extremes of variation were selected to analyze their bone modeling patterns. Table 1 summarizes the data on age and sex of the analyzed individuals, as well as the presence of bone formation and resorption areas for the studied regions. Figure 5 shows the bone modeling maps for the glabella, the superciliary arch and the malar of each individual, showing scattered bone modeling fields that vary in shape and size. It is worth noting that all individuals present areas without histological information due to alteration of the bone surface; the most extreme case corresponds to individual Ch1098 from Chubut whose bone surface is completely altered. Follows a detailed description of the bone modeling patterns obtained for each anatomical region.

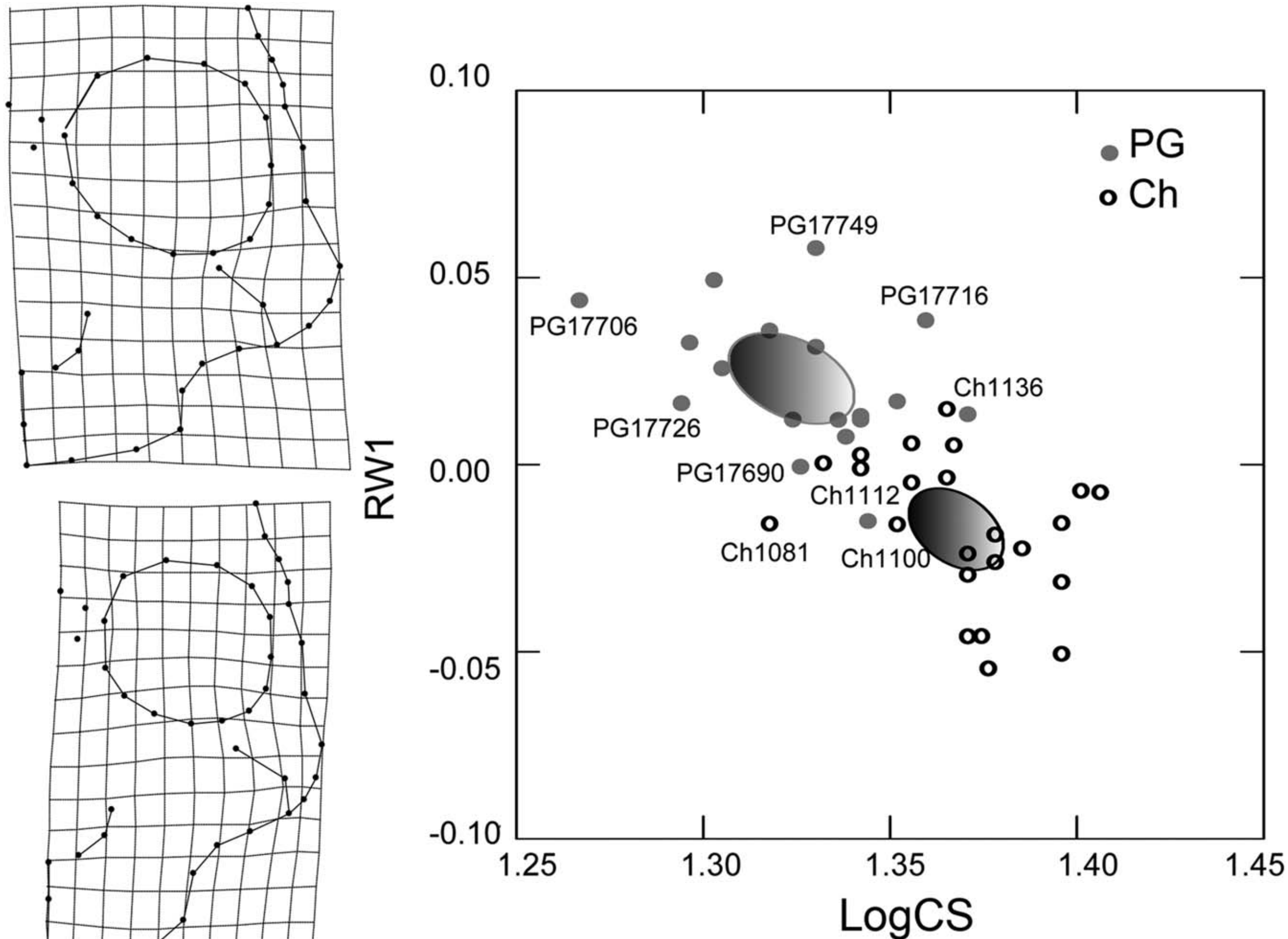

Fig. 4. Scatterplot of Chubut (Ch) and Pampa Grande (PG) individuals in the form space represented by Relative Warp 1 (RW1) and the log-transformed centroid size (Log CS) of the facial skeleton in frontal view. The deformation grids illustrate the shape changes at the negative and positive extremes of the first axis (RW1).

Malar. The pattern of the malar bone is characterized by the presence of large bone formation fields and absence of bone resorption. In the Pampa Grande sample, the malar region of individual PG17749 presented bone formation fields all over its surface, while in the rest of the sample, such fields were identified in certain areas of this region. The individuals PG17726, PG17706, and PG17716 show formation fields on the ascending ramus and the center of this region around the zygomatic foramen, extending over the orbital margin and the upper margin of the zygomatic ramus. In individual PG17726, these fields extend to the zygomatic-maxillary suture. Individual PG17690 is quite altered and shows only small formation areas at the frontal-zygomatic suture and the upper margin of the zygomatic ramus. Regarding the Chubut sample, only individual Ch1112 shows bone formation fields, whereas it was not possible to identify histological features in the remaining individuals because of altered surfaces. In Ch1112, bone formation was identified along the orbital margin, in the central portion, and along the zygomatic-maxillary suture.

Glabella. Although this region shows high degree of alteration, at least two individuals from Pampa Grande (PG17749 and PG17690) and one from Chubut (Ch1136) have preserved fields of bone modeling that show the dynamics of this region. In the case of the Pampa Grande sample, individual PG17749 presents bone formation fields over the entire region and a large bone resorption field in the upper part of the glabella. The individual PG17690 shows two small resorption fields in the upper glabella. The Chubut individual Ch1136 presents only formation fields distributed in the upper and lower halves of the glabella.

Superciliary arch. This region presents both bone formation and resorption fields. In the Pampa Grande sample, individual PG17749 shows bone formation fields all over this region, while PG17706 shows only two small fields, one near the lower orbital margin and the other close to the frontal-zygomatic suture. In addition, individual PG17706 presents bone resorption fields in the central part of this region. The individual PG17726 also shows small resorption fields in the upper part of

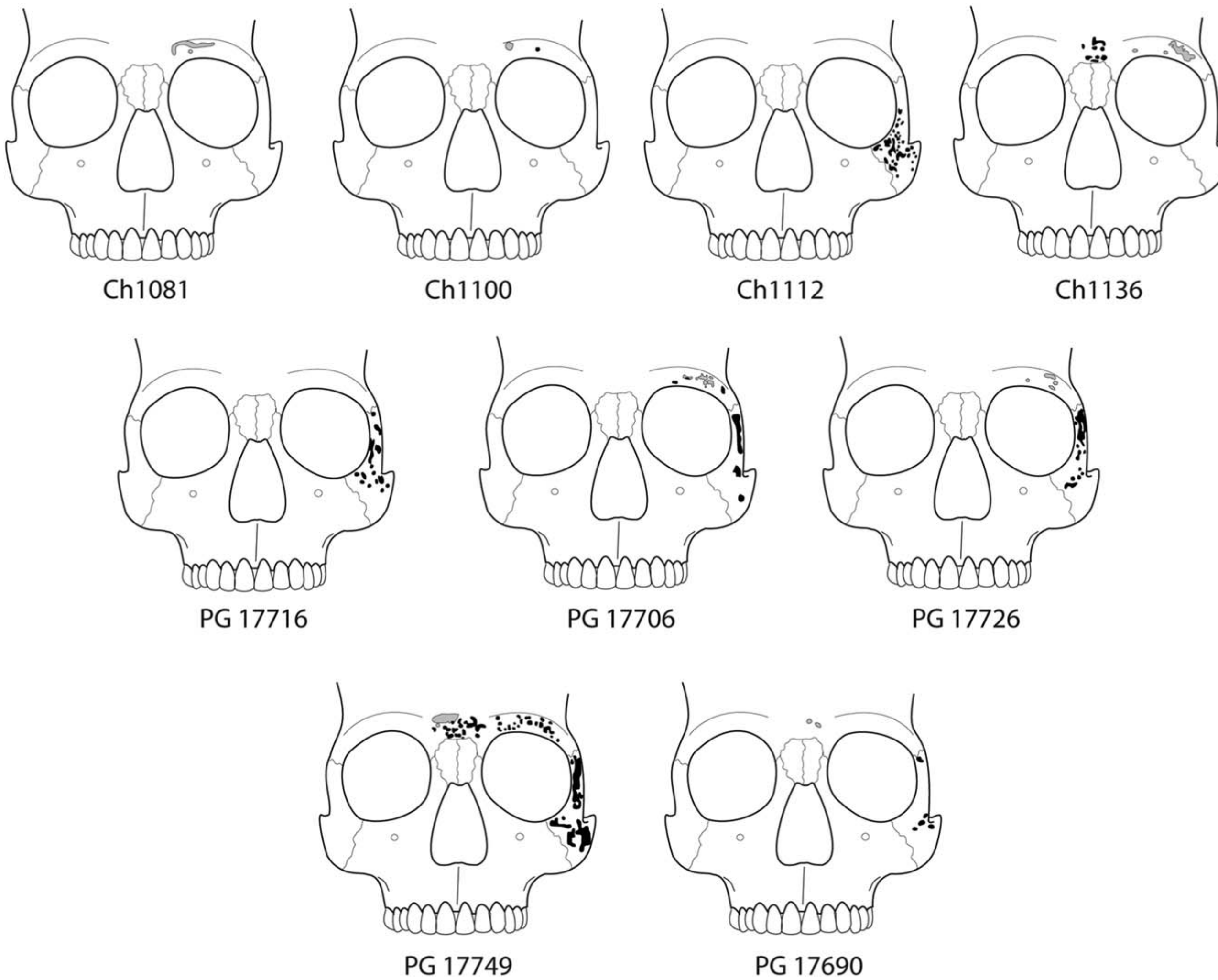

Fig. 5. Bone modeling maps. Black: bone formation; gray: bone resorption; white: damaged bone surfaces with no histological data.

this region. The Chubut sample is characterized by having resorption fields located in the upper region near the glabella (Ch1081), in the area close to the frontal-zygomatic suture, and near the supraorbital margin (Ch1136). The individual Ch1100 shows two small fields, a bone formation field in the center of this region and a bone resorption one in the upper area, next to the glabella.

DISCUSSION

Craniofacial morphological variation is related to differences in the distribution of bone formation and resorption fields that indicate different growth dynamics (e.g., Bromage, 1989; Enlow and Hans, 1996; McCollum, 2008; Martinez-Maza et al., 2013; Lacruz et al., 2013). Until now, studies on this subject have shown the existence of a particular bone modeling pattern for each species, but its role regarding intraspecific variation has been scarcely studied (McCollum, 2008; Martinez-Maza et al., 2013). In this sense, the present work represents a first approach to the study of the cellular mechanisms involved in the morphometric variation of human populations from southern South America. This study is highly interesting for studies of craniofacial morphology in general, because it is the first analysis of the morphological variation of a single sample by two integrated complementary approaches, i.e., geometric morphometric analysis and study of bone modeling patterns.

The morphometric analysis of the facial structures of Pampa Grande and Chubut samples agrees with the general pattern documented for South American populations, with larger size and stronger development of the glabella and superciliary arch in the hunter-gatherer adults than in the farmers (Sardi et al., 2005; Perez and Monteiro, 2009). A reduction in size and robusticity of cranial traits associated with an increased consumption of domesticated plants has been found in other geographic regions, although the patterns seemed to vary according to the populations being as compared (Carlson and Van Gerven, 1977; Paschetta et al., 2010; von Cramon-Taubadel, 2011). On the other hand, the analysis of bone surface allowed to characterize the distribution of bone formation and resorption fields in the three facial structures studied here. In particular, the bone modeling of the malar bone showed a common pattern in all individuals characterized by the presence of bone formation areas. Conversely, the glabella and the superciliary arch presented both formation and resorption areas, but the distribution of the respective fields varied between the Pampa Grande and Chubut individuals.

The relationship between facial variation summarized in the form space and the bone modeling maps suggests that the differences in malar form among individuals would not be attributable to variations in the distribution of bone formation and resorption areas, because all the individuals showed bone formation. Consequently, the larger size of the malar and the development of its frontal and zygomatic processes in Chubut individuals would be linked to greater magnitude of bone formation activity. Unbalance favoring bone formation results in an increase of size during growth (Enlow and Hans, 1996), and therefore, differences in bone formation rates could explain the size and shape variation among adult individuals. The glabella and the superciliary arch displayed greater disparity in the patterns of bone modeling among individuals. In the extreme condition corresponding to the more robust individuals from the Chubut population, the great development of the glabella is related to the presence of large formation fields, both in the upper region and toward the frontonasal suture. On the contrary, the less robust morphologies from Pampa Grande show resorption fields at the upper margin of the glabella, which would contribute to the weaker development of this region. The superciliary arch display a complex relationship between its morphometric and histological variation, since the individuals at both extremes of the shape space present large resorption areas on the upper margin of this structure.

The combined analysis of facial morphometric variation at anatomical level and of bone modeling patterns performed here contributes to the discussion regarding the mechanisms responsible for the variation observed among adult individuals. Previous research on native South American populations were aimed at establishing whether the shape and size differences between populations entailed changes in allometric trajectories, in the age of cessation of growth, or in the rate of growth (González et al. 2010, 2011; Barbeito Andrés et al., 2011). The presence of bone formation fields in the malar of adult individuals of similar age that differ markedly in the size of this structure suggests that such morphological differences would have more probably resulted from variation in the rate of bone formation, rather than from the prolongation of bone formation activity. In this sense, the larger size of the masticatory component in hunter-gatherers since early ontogenetic stages (Barbeito Andrés et al., 2011) also supports the hypothesis that differences in growth rate would account for interpopulational variation.

Assessing whether the pattern of bone modeling described here is a particular feature of the adult individuals of the populations under study, or reflects the variability of the species, requires the comparison of a larger number of samples. The scarcity of studies of craniofacial bone modeling in adult *Homo sapiens* restricts comparison of the results obtained here. Until now, the only reference about variation in the bone modeling pattern of facial structures is the work by Martinez-Maza et al. (2013). The sample analyzed by these authors comes from the anthropological collection of Identified Skeletons belonging to Universidade de Coimbra (Portugal) consisting of individuals dated between the late 19th century and early 20th century (Matos Fernandes, 1985). This collection has detailed information for each individual (age, sex, employment, cause of death, and geographical origin). Comparison of the present results with those from the Coimbra sample indicates that the bone modeling patterns of the glabella and superciliary arch of Chubut and Pampa Grande resemble those recorded for the adult sample in Coimbra. Unlike the condition observed in South American individuals, the pattern of the malar in the Coimbra sample displays bone resorption along its entire lower margin and its temporal process up to the temporal-zygomatic suture, as well as on the infraorbitary margin in the area of contact between glabella and superciliary arch.

Taking into account observations from previous studies (Kurihara et al., 1980; Enlow and Hans, 1996; McCollum, 2008), differences observed in the facial regions may be attributable to environmental factors or to the evolutionary history of populations. Particularly, the mechanical loads exerted on bone tissue are among the most important factors to stimulate bone formation (Robling et al., 2006); thus, the structures directly involved in food processing are expected to present more extensive bone formation fields. Numerous studies have shown that the zygomatic arch region is strongly influenced by forces exerted during mastication, whereas stress is very low on the glabella and superciliary arch (Hylander and Johnson, 1997; Ravosa et al., 2000; Vinyard and Smith, 2001; Ross and Metzger, 2004; Wroe et al., 2010; Athreya, 2012). This could explain the predominance of bone formation in the malar compared to the other structures analyzed, as well as the differences in modeling patterns of malar bone between the Coimbra and South American samples. On the other hand, the differential hardness of consumed foods would not be enough to explain the degree of robusticity of the supraorbital region. As an alternative hypothesis, a positive association between skull size and development of this region has been suggested, which would be related to systemic factors (e.g., increase in circulating growth hormone) that result in greater cortical robusticity (Lieberman, 2011; Athreya, 2012). Such factors have been previously proposed to explain differences in robusticity between South American populations (Bernal et al., 2007).

Our goals for the future include more in-depth studies through the histological analysis of ontogenetic, in order to describe the changes in bone modeling that take place during development. The combination of these data with the description of morphological changes from three-dimensional morphometric analyses will allow the generation of development models and the assessment of hypotheses regarding the ontogenetic mechanisms involved in interpopulational variation.

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