

Morphological Integration of the Orbital Region in a Human Ontogenetic Sample

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ABSTRACT

Most studies on craniofacial morphology have focused on adult individuals, but patterns of variation are the outcome of genetic and epigenetic variables that interact throughout ontogeny. Among cranial regions, the orbits exhibit morphological variation and occupy an intermediate position between neurocranial and facial structures. The main objective of this work was to analyze postnatal ontogenetic variation and covariation in the morphology of the orbital region in a cross-sectional series of humans from 0 to 31 years old. Landmarks and semilandmarks were digitized on the orbital rim, as well as in neighboring neural and facial structures. Data were analyzed using geometric morphometrics. Results indicated that orbital size increases during the first years of postnatal life, while the shape of the orbital aperture does not change significantly with age. In general, the pattern and magnitude of shape covariation do not vary markedly during postnatal life although some subtle shifts were documented. Additionally, the shape of the orbital aperture is more related to the anterior neurocranium than to zygomatic structures, even when the allometry is adjusted. Although we expected some influence from postnatal craniofacial growth and from some functional factors, such as mastication, on the development of the orbits, this assumption was not completely supported by our results. As a whole, our findings are in line with the prediction of an early influence of the eyes and extraocular tissues on orbital morphology, and could be interpreted in relation to processes promoting early neural development that coordinately affects orbital traits and the neurocranial skeleton. *Anat Rec*, 299:70–80, 2016. © 2015 Wiley Periodicals, Inc.

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INTRODUCTION

Craniofacial morphological variation has been the focus of several works in physical anthropology that aim to understand which evolutionary processes were involved in the diversification of modern humans (e.g., Relethford, 1994; Hennessy and Stringer, 2002; Roseman, 2004; Sardi et al., 2005; Bernal et al., 2006; Perez and Monteiro, 2009) and other primates (e.g., Ackermann, 2002; Athreya, 2012; Rocatti et al., 2014). The orbits exhibit morphological variation in human populations, and their examination has been useful to detect taxonomic and functional differences between primates (Kay and Kirk, 2000; Lieberman, 2011).

Some works focusing on our species have found significant variation in orbital morphology among populations from different geographic regions, such as South America (Lahr, 1995; Perez et al., 2007; Barbeito-Andrés et al., 2011), Europe (Sardi et al., 2004), Africa, and Asia (Masters, 2008). While certain authors have suggested that the orbital region shows some of the highest heritability values of the human skull (Martínez-Abadías et al., 2009a), it has also been stated that the morphology of the orbit is not strongly related to genetic data and, therefore, it is not reliable to study populational history from its shape variation (von Cramon-Taubadel, 2011).

Studies that assess phenotypic variation between and within populations are usually based on adult individuals. However, adult morphology is the result of particular ontogenetic trajectories in which different factors and processes interact (O'Higgins and Jones, 1998; Strand and O'Higgins, 2000). Therefore, the analysis of ontogenetic patterns in the orbital region may explain some aspects of the sources of morphological variation in human populations.

Although the human orbit is usually considered as an anatomical unit, it is actually formed by seven different bones: ethmoid, sphenoid, frontal, zygoma, maxilla, palatine, and lacrimal. The multiple sutures linking these bones allow the orbits to grow in several and diverse directions (Enlow and Hans, 1996; Lieberman, 2011). Enlow and Hans (1996) proposed that orbits grow under the "V-principle": the conical orbital cavity drifts forward to its opening as bone deposition occurs on the inside. Through this mechanism, orbital volume increases, rather than decreases, while the orbital rim also enlarges (Enlow and Hans, 1996). According to the functional matrix hypothesis, the content of the orbit (eyeball and extraocular tissues) is the variable that most influences its growth (Moss and Young, 1960; Cepela et al., 1992). Eyeballs grow rapidly during early ontogeny and they are thought to exert a strong stimulus on orbital morphogenesis (Sarnat, 1982; Goldstein et al., 1998; Tomasik et al., 2005). However, the growth of the orbit and its contents is decoupled through much of ontogeny and the eyeballs produce a limited effect on postnatal orbital growth (Schultz, 1940; Washburn and Detwiler, 1943; Hoyte, 1997; Sperber, 2001; Cumminngs et al., 2012). In this line, some authors have found a moderate influence of the eyes on orbital morphology, suggesting that the key role of eyeball growth should not be straightforwardly assumed for the whole ontogeny (Masters, 2008; Smith et al., 2014). Hence, the interaction with other growing regions during postnatal life may be relevant.

The orbits are structurally placed in an interface position between the neurocranium, the nasal cavity, the maxillary sinus, and the temporal fossa. Relations between the orbits and other craniofacial structures, as well as their developmental link with neighboring tissues and organs, suggest that morphological integration is a particularly interesting aspect of this anatomical region to be studied. Morphological integration, which refers to the coordinated change in developmentally or functionally related traits, constrains or promotes morphological change in certain directions (Olson and Miller, 1958; Hallgrímsson et al., 2002; Klingenberg, 2004, 2005; Martínez-Abadías et al., 2009b).

In this work, we analyzed variation in the size and shape of the orbital region in an ontogenetic series of human individuals from 0 to 31 years old. On the basis of several studies on fetal and early postnatal development, it is predicted that the morphological changes in the orbit during this period are driven by the growth of the eyeball and other soft tissues it contains. It is also known that some aspects of craniofacial morphology, especially in the face, continue to change for an extended period of time during postnatal life (Buschang et al., 1983; Enlow and Hans, 1996). Thus, it might reasonably be expected that the orbits will be influenced by these later dynamics, particularly by some functional factors, such as mastication. Before weaning, mastication may have a reduced effect, but afterward this factor impacts strongly on facial morphology and could modify the orbit once eye growth is completed.

Under the prediction of an initial influence of the growing eye, it is feasible to observe early-established patterns of covariation among traits. Moreover, if craniofacial growth affects the orbits at later stages of ontogeny, integration patterns would vary postnatally. If this was the case, a lower magnitude of integration should be observable in latter developmental stages when the effect of various growing structures and functional factors overlaps after the eyeballs have stopped growing.

Among the neighboring structures, an important relation between the orbital aperture and the anterior neurocranium is expected since the frontal lobes are placed on the roof of the orbits and both orbital and neurocranial structures interact with neural tissues and organs (Sperber, 2001). Adjacent facial regions would also show some coordinated variation with the orbits. In particular, the relation with zygomatic morphology, which is influenced by masticatory dynamics, is a relevant example to be examined (Lieberman et al., 2004; Paschetta and González-José, 2012). It is worth noting that even though some midfacial structures, such as the nasal cavity and maxilla, are adjacent to the orbits and structural relations among their traits are expected, these regions could not be analyzed because images of the most inferior part of the midface were not available for many of the subjects in our database. Here, covariation with the anterior neurocranium and the zygomatic region was evaluated to discuss to what extent the shape of the orbital aperture develops in coordination with neural and facial neighboring structures.

In summary, through this comprehensive analysis based on a wide range of postnatal ages, we aimed to discuss how the generation of adult craniofacial phenotypes would be constrained by some developmental processes.

TABLE 1. Sample composition by age group and sex.

<i>n</i> total	<i>n</i> infant-child	<i>n</i> juvenile	<i>n</i> adolescent	<i>n</i> adult
122	16 (F = 6, M = 10)	21 (F = 7, M = 14)	22 (F = 9, M = 13)	63 (F = 45, M = 18)

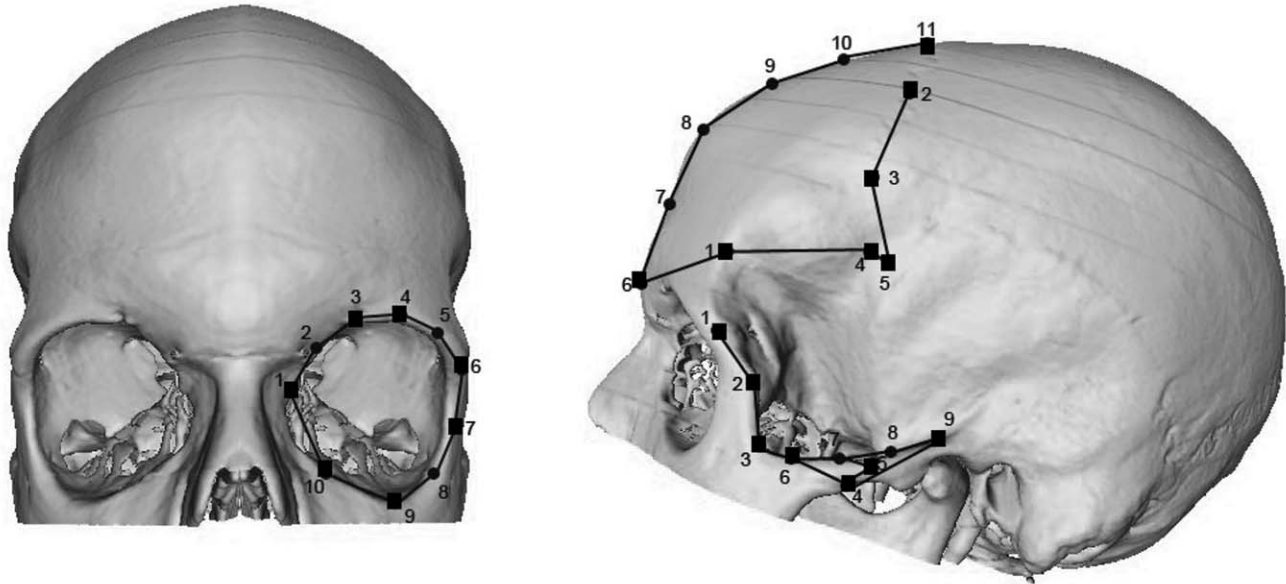


Fig. 1. Landmarks and semilandmarks digitized on the orbital region, anterior neurocranium, and zygomatic region. Squares represent landmarks, and circles correspond to semilandmarks. Wireframes linking points are introduced as they are used in the following figures.

MATERIALS AND METHODS

The Sample

Analyses were carried out on a dataset of head computed tomography (CT) images constructed at the medical institution Fundación para la Lucha contra las Enfermedades Neurológicas de la Infancia in Buenos Aires, Argentina. The sample consisted of 122 living individuals from 0 to 31 years old of both sexes with no neurological pathologies (Table 1). They were scanned with a General Electric LightSpeed RT16 and, for each individual, a set of 208–304 axial CT images with a resolution of 512×512 pixels and a voxel size of $0.449 \times 0.449 \times 0.625$ mm was produced. To examine the CT images and create reconstructions, a trial version of Avizo 6.0 software (Visualization Science Group) was used. Reconstructions in three dimensions (3D) were obtained using an empirically chosen threshold of 1150 Hounsfield units to show the maximum amount of bony tissue.

Some statistical analyses required individuals to be divided into age groups (Table 1). For this purpose, we chose a scheme based on important life-history events (Bogin, 1999) and distinguished infant-child (0–6 years), juvenile (7–12 years), adolescent (13–17 years), and adult (18–31 years).

Data Collection and Statistical Procedures

Geometric morphometric techniques were used to assess morphological variation. A set of landmarks and

semilandmarks was digitized on the left side of each skull to describe the shape of the orbital aperture, of the anterior neurocranium and the zygomatic region (Fig. 1 and Table 2). Semilandmarks were replaced along the outline curve using a linear interpolation between the original curve points (Reddy et al., 2004). Generalized Procrustes superimpositions were performed on each set of landmarks and semilandmarks and, as a result, one variable of size (centroid size) and a set of shape variables (Procrustes coordinates) were obtained (Rohlf and Slice, 1990). An estimation of the volume of the orbit was obtained through semiautomatic segmentation of the whole cavity. For further analyses, centroid size and orbital volume were transformed into their natural logarithms (nlCS and nlOrbV, respectively).

Prior to definite digitization of landmarks and semilandmarks, an intraobserver error analysis was carried out to estimate differences among the measurement events performed by the author who collected the data (J.B.A.). Measurement error was assessed by means of different statistical approaches described elsewhere (Barbeito-Andrés et al., 2012). In general, the landmarks and semilandmarks used here (for the orbital rim, anterior neurocranium and zygomatic region) were not problematic when error assessment was carried out; this is especially true for type I landmarks, which are defined by anatomical accidents such as the intersection of sutures (Barbeito-Andrés et al., 2012).

As the first step, the relation between age and size variables (nlCS and nlOrbV) was described using

TABLE 2. Landmarks and semilandmarks recorded.

Region	Number ID	Denomination
Orbit	1	Dacryon
Orbit	2	Semilandmark
Orbit	3	Optic foramen
Orbit	4	Supraorbitale
Orbit	5	Semilandmark
Orbit	6	Anterior fronto-malare
Orbit	7	Ectoconchion
Orbit	8	Semilandmark
Orbit	9	Infraorbitale
Orbit	10	Zygoorbitale
Zygomatic region	1	Malar frontotemporale
Zygomatic region	2	Jugal prominence
Zygomatic region	3	Jugal
Zygomatic region	4	Inferior zygotemporal
Zygomatic region	5	Zygion
Zygomatic region	6	Superior zygotemporal
Zygomatic region	7	Semilandmark
Zygomatic region	8	Semilandmark
Zygomatic region	9	Posterior zygomatic
Anterior neurocranium	1	Frontotemporale
Anterior neurocranium	2	Superior staphanion
Anterior neurocranium	3	Inferior staphanion
Anterior neurocranium	4	Sphenion
Anterior neurocranium	5	Pterion
Anterior neurocranium	6	Glabella
Anterior neurocranium	7	Semilandmark
Anterior neurocranium	8	Semilandmark
Anterior neurocranium	9	Semilandmark
Anterior neurocranium	10	Semilandmark
Anterior neurocranium	11	Bregma

scatterplots. Then, we evaluated the relation between the size of the orbit and the aperture shape by performing multivariate regression using nlCS and Procrustes coordinates as independent and dependent variables, respectively. Shape variation was evaluated through Principal Components Analysis (PCA) of Procrustes coordinates. A new set of variables, the principal components (PCs), was obtained. Each PC captures main directions of shape variations.

Then, ontogenetic changes in the patterns of morphological integration were assessed. Here, pattern refers to the structure of relationships between morphological traits that can be studied by examining the associations among traits (Porto et al., 2009). Covariance matrices of orbital landmarks and semilandmarks were estimated for each age group. To control the effect of sexual dimorphism, we pooled within-sex covariance matrices. Afterward, the matrices for each age group were compared using three approaches. First, we computed correlations between pairs of matrices, where the strength of the association between matching elements of both matrices was assessed. Correlation values can range from 1 (matrices are equal) to -1 (matrices are mirror images). If the value is 0, it indicates that matrices are structurally unrelated (Marroig and Cheverud, 2001). Due to potential differences in the patterns of variation among groups, matrix correlations were adjusted using the formula described by Marroig and Cheverud (2001), where $R_{adj} = R_{obs}/R_{max}$. R_{max} represents the maximum matrix correlation, which was obtained through the formula $R_{max} = \sqrt{(t_a \times t_b)}$. Matrix repeatability (t) was estimated by resampling the original data sets 1000 times and cal-

culating the covariance matrices again to obtain a mean matrix correlation (Marroig and Cheverud, 2001). Similarity between matrices was also assessed through random skewers vector correlation, which compares the response of covariance matrices to a random selection vector (Cheverud, 1996). Finally, a distance between two covariance matrices was calculated following Mitteroecker and Bookstein (2009). According to this measure (MB distance), similarity between matrices should be analyzed without focusing on significance tests, but exploring the distance on the space of positive-definite symmetric matrices. MB distances were calculated using matrices obtained from the first 11 PCs (this number corresponds to the number of landmarks and, in all cases, the first 11 PCs explained more than 90% of variation) as the square root of the summed squared logarithms of the relative eigenvalues between compared matrices (Mitteroecker and Bookstein, 2009).

The magnitude of covariation (intensity or degree of association among traits) in each age group was assessed using the variance of eigenvalues (Pavlicev et al., 2009). In cases of strong covariation, the variance of eigenvalues is high and it is low when covariation among traits is weak. With the aim of comparing this measure of covariation degree across groups, the variance of eigenvalues was scaled in each case by the total shape variance within each group, following Young (2006). Ranges of scaled variance of eigenvalues were obtained from resampling with replacement (1000 iterations).

Morphological integration between the orbital aperture and other anatomical structures (anterior neurocranium and zygomatic region) was explored with two-block Partial Least Squares (PLS) analysis. New linear combinations of shape variables that more accurately represent covariation between blocks of variables were obtained (Rohlf and Corti, 2000; Bookstein et al., 2003). To assess the impact of size change on covariation among craniofacial regions, PLS analyses were repeated using the residuals of multivariate regression of Procrustes coordinates on CS. This procedure is based on the fact that shape information that is not predicted by the regression model is retained in the residuals and provides a frame to evaluate covariation once the effect of size on shape is adjusted (Monteiro, 1999; Klingenberg, 2009).

All morphometric and statistical analyses were performed using MorphoJ (Klingenberg, 2011) and R (R Core Team, 2012).

RESULTS

Orbital Size and Shape Variation during Postnatal Life

Size variables (nlOrbV and nlCS) increased strikingly during the first year of postnatal life and then the growth was more gradual until the age of 6 approximately (Fig. 2). Although nlOrbV and nlCS represent changes in different structures, ontogenetic variation was similar in both size variables.

According to multivariate regression, nlCS explained only 2.92% of shape variation of the orbital aperture. Although this result is significant ($p = 0.0008$), it is notable that the proportion of allometric variation is small. The first three PCs explained more than 50% of

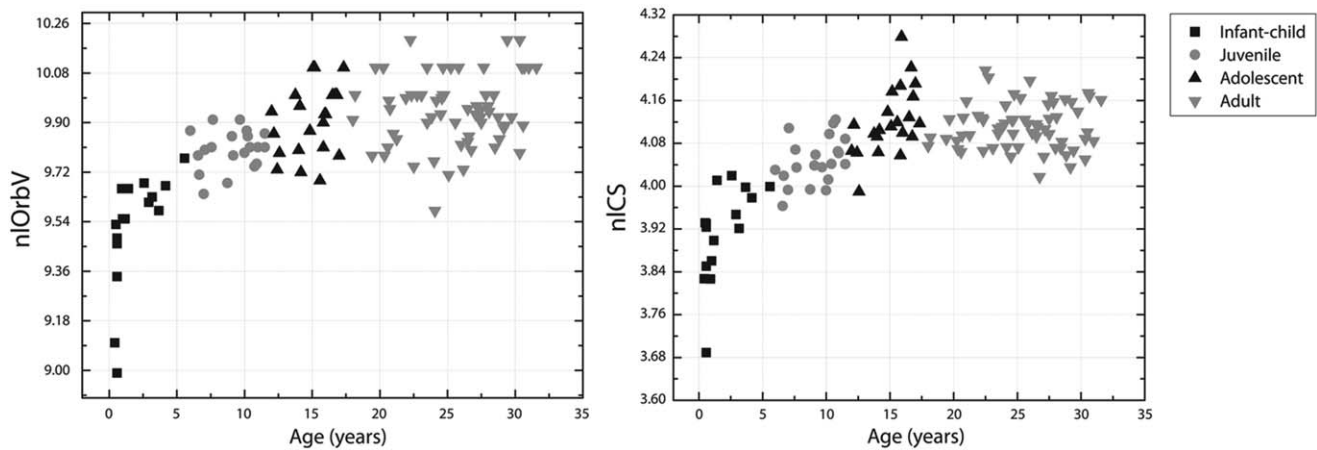


Fig. 2. Size changes in the nICS of the orbital rim and the nOrbV during postnatal life.

total shape variation. Correlations between these PCs and age and size (nICS) were assessed by the calculation of Pearson correlation coefficients. None of the PCs were significantly correlated with age or nICS ($p < 0.05$). In this line, the distribution of individuals along PC1 and PC2 shows that there is no clear pattern of ontogenetic change (Fig. 3). Across PC1, there were noticeable shape differences in the medial points, while in PC2, shape changes were present in different parts of the orbital rim, being especially marked in the uppermost point of the orbit (landmark 3). However, this shape variation does not represent ontogenetic changes (Fig. 3).

Ontogenetic Change in Patterns and Magnitude of Covariation

To test the similarity of covariation patterns in the orbital aperture among ontogenetic stages, different analyses were performed. Matrix correlations showed a highly significant association between all of them. Correlation values ranged from 0.679 to 0.841, and they increased slightly when adjusted (Table 3). Similarly, all correlations were highly significant for the random skewers vector correlations, which ranged from 0.630 to 0.786 (Table 4). Although the correspondence is stronger between adolescent and adult groups, the high correlations found in all the cases suggest that there is no remarkable change in covariation patterns during ontogeny.

Through the analysis of MB distance, similarity between matrices can be observed in relative terms, making it possible to identify which ones display the longest distances and which ones are nearer. Here, we found that the adult group is the most differentiated as regards its covariance matrix, in particular, when compared with the infant-child group. On the other hand, the shortest distance was found between the juvenile and adolescent groups (Table 4).

Regarding the strength of covariation, the scaled variance of eigenvalues was similar in all age stages (Fig. 4). Although the highest value was observed for the juvenile group and the lowest for adults, bar graphs with standard deviation overlapped across most of the

ages. Therefore, results indicated that there is a relative stability of the magnitude of covariance during postnatal life.

Covariation with Other Craniofacial Regions

To explore the relation between the shape of the orbital aperture and other craniofacial regions, two-block PLS analyses were conducted. For the orbit and the anterior neurocranium, PLS1 accounted for 76.62% of total covariation (Table 5). Along the PLS1, infants-children were placed at the negative scores, juvenile individuals at an intermediate position, while the adolescent and adult groups overlapped at the opposite positive extreme (Fig. 5). The distribution of individuals along the axes reflects that orbit shape changed markedly during the first ontogenetic stage, while the anterior neurocranium showed a more moderate variation. However, for most of their postnatal life, both structures seemed to vary coordinately (Fig. 5). In the negative scores, the sagittal profile of the anterior neurocranium was higher, co-varying with changes in the medial and superior margins of the orbit (Fig. 5). In advanced stages of postnatal life, the dacryon showed a more medial position, and the landmark corresponding to the orbital foramen changed towards a relatively inferior place, while the neurocranial region was vertically shorter and with a more projecting glabella (Fig. 5). When the nICS-adjusted PLS was carried out, covariation between the anterior neurocranium and the orbit summed by the PLS1 decreased by about 10%, yet it remained significant.

Concerning covariation with the zygomatic region, the PLS1 explained 50.33% of covariation (Table 5). Infants-children were partially separated from the other groups, which overlapped clearly (Fig. 6). In young individuals located at the positive extremes of PLS1 distribution, a relatively more anteriorly placed jugal covaried with subtle changes in the medial, lateral, and superior parts of the orbital aperture. Toward negative scores, which better represent adults, the height of the zygomatic arch was increased and the zygion was more posteriorly placed (Fig. 6). After adjusting the effect of allometry,

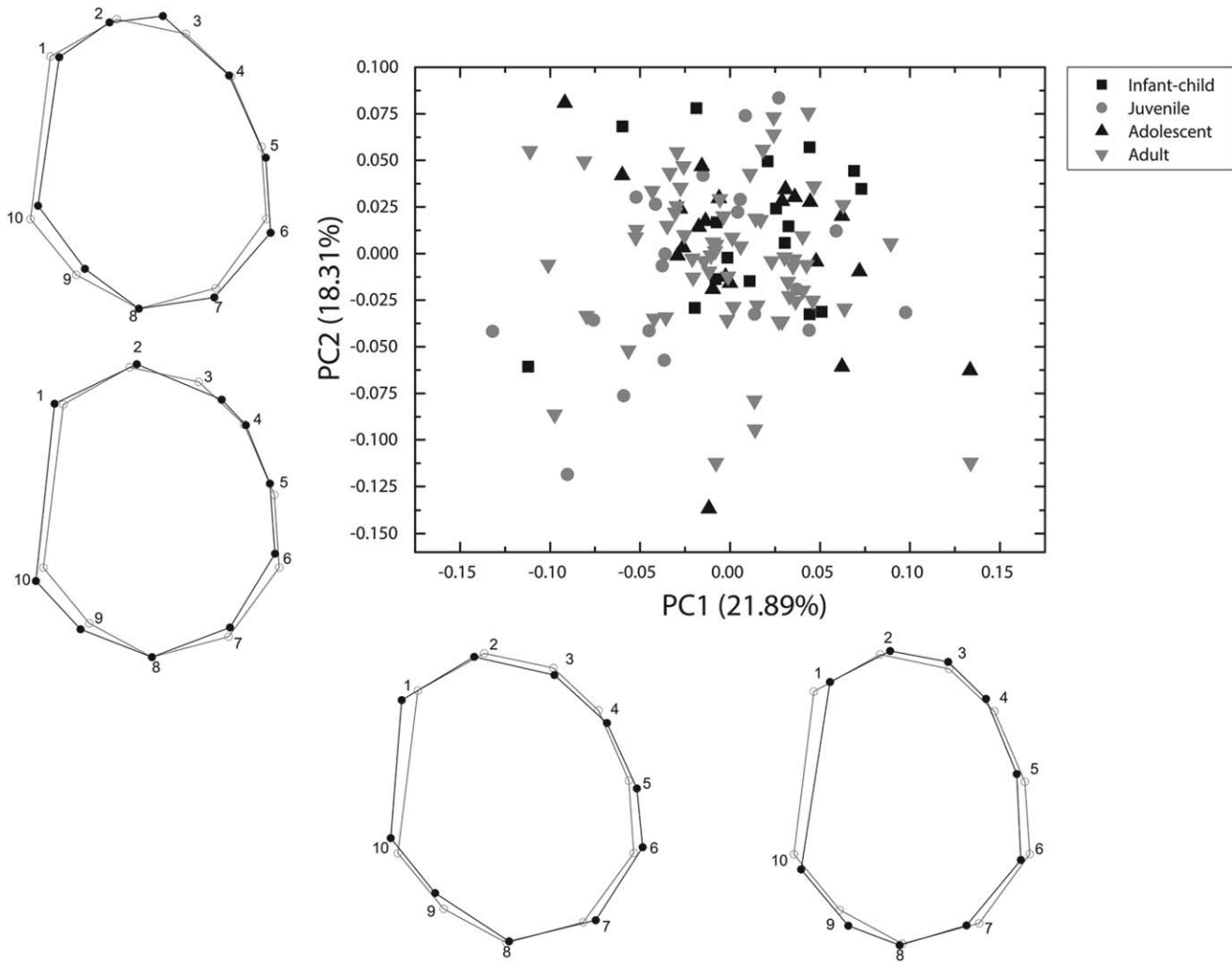


Fig. 3. PC1 vs PC2 for orbital region. Black wireframes show extreme shape configurations, and grey wireframes represent the mean shape.

TABLE 3. Correlation matrix for each ontogenetic stage pairwise comparison for the orbital rim.

	Infant-child	Juvenile	Adolescent	Adult
Infant-child	0.9051	0.7217**	0.6787**	0.7231**
Juvenile	<i>0.7929</i>	0.9154	0.7197**	0.7517**
Adolescent	0.1712–0.3139 <i>0.7548</i>	<i>0.7959</i>	0.8932	0.8410**
Adult	0.1983–0.3404 <i>0.8097</i>	0.2158–0.3808 <i>0.8369</i>	<i>0.9479</i>	0.8812
	0.2355–0.3595	0.2641–0.4060	0.2563–0.3834	

Matrix repeatability (*t*) in the diagonal, with raw correlations in bold in the upper triangle of the matrix and adjusted correlations (italic) in the lower triangle of the matrix. Above adjusted values, 95% confidence limits estimated by bootstrap resampling. **p* > 0.01, ***p* > 0.0001.

covariation between both blocks became weaker and the PLS1 accounted for 28.04% of covariation, being not significant (Table 4).

In general, PLS results indicated that the orbital aperture was more related to the anterior neurocranium than to zygomatic structures, even when the allometry was adjusted.

DISCUSSION

While many studies have focused mainly on adult morphology, patterns of variation are the outcome of several genetic and epigenetic variables that interact throughout ontogeny. In this context, studies that specifically evaluate some aspects of morphological change

TABLE 4. Random skewers vector correlation for each ontogenetic stage pairwise comparison above and corresponding probabilities estimated by bootstrap resampling. MB distance for each pairwise stage comparison in the lower half.

	Infant-child	Juvenile	Adolescent	Adult
Infant-child	1	0.6301 (<0.0001)	0.6303 (<0.0001)	0.6748 (<0.0001)
Juvenile	1.0494	1	0.6868 (<0.0001)	0.7055 (<0.0001)
Adolescent	1.2984	0.8901	1	0.7861 (<0.0001)
Adult	2.3472	1.572	1.98	1

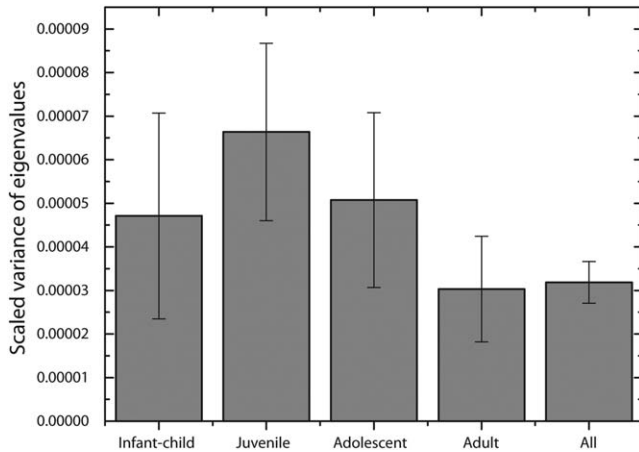


Fig. 4. Scaled variance of eigenvalues for each ontogenetic stage. Standard deviations obtained after resampling (1000 iterations) are represented by the error bars.

during different developmental stages are useful to interpret the processes involved in the generation of phenotypic diversity. The main objective of this work was to analyze ontogenetic variation and covariation in the morphology of the orbital region to discuss processes that may have significance during postnatal life. According to our results, orbital size increases during the first years of postnatal life, while the shape of the orbital aperture does not change significantly with age. This stability throughout ontogeny is also observed in the pattern and magnitude of covariation among orbital shape traits, which show coordinated variation with neurocranial structures. In general, these results are consistent with the expectation of an important influence of the eyes on orbital morphology during prenatal and early postnatal ontogeny.

As stated by Enlow and Hans (1996), we found that the expansion in the volume of the orbital conical cavity (nlOrbV) is coordinated with the enlargement of the orbital aperture (nlCS). This fact is evident from the similarity in ontogenetic trajectories between both size variables (Fig. 2). Morphological variation (not only size but also shape) has been related to the development of functional matrices; in the case of our study, mainly the eyeballs and extraocular tissues. This is in agreement with experimental studies that have demonstrated that immature osseous structures of the orbit respond to the stimulus of orbital implants (Cepela et al., 1992; Wagner et al., 2000) and that there is an early period when the eye strongly influences adjacent structures, followed by a time when the orbit is already ossified and changes

limitedly (Washburn and Detwiler, 1943). Early morphogenesis of the orbital content would constrain orbital rim shape, which showed here a remarkable stability during the studied age range. The observed stability in the pattern and magnitude of covariation during postnatal life is also in line with the assumption of an early influence of the functional matrix that intervenes in the establishment of relations among orbital traits. As a complement to the Functional Matrix Hypothesis, a gradient of maturation has been suggested for craniofacial structures, where upper regions develop earlier than the middle and lower face (Buschang et al., 1983; Enlow and Hans, 1996; Sardi and Ramirez-Rozzi, 2005; Bastir et al., 2006). Although the floor and walls of the orbit are formed by facial bones, which take longer than neural structures to complete maturation, the orbital aperture as a whole seems to be only slightly affected by these facial dynamics.

Further results of this work indicated that the orbital aperture is developmentally more related to the neurocranium than to facial organs and tissues. In particular, its shape traits covaried strongly with the anterior neurocranium during postnatal life, even when the effect of allometry was adjusted (Table 5). Although the orbits occupy an intermediate position, Sardi and Ramirez-Rozzi (2005) detected that the growth trajectory of the volume of the orbital cavity is more similar to the trajectories of neurocranial components than to those of facial components. In cross-population comparisons, Sardi (2002) and Sardi et al. (2004) observed that populations with relatively greater neurocranial cavities also exhibited greater orbital cavities. This similarity may occur because the eyeball and the brain present, to some extent, a common embryological origin and both display early patterns of development (Sardi, 2002; Sardi et al., 2004; Sardi and Ramirez-Rozzi, 2005). Certain structural relationships are likely to have a role as well since the roof of the orbit is also the floor of the anterior cranial fossa (Lieberman, 2011). The congruence in the ontogenetic patterns of neurocranial and orbital regions could be interpreted as driven by common developmental processes promoting neural development. According to our results, when considering the individuals at the negative extreme of the distribution in the PLS analysis (Fig. 5), orbital variation seems to be partially unrelated to the anterior neurocranium during early life. This could be explained by the very early and more prominent influence of the eye and a subsequent more prolonged relation with other craniofacial structures. However, to properly confirm or reject this two-stage pattern for orbital development, it would be necessary to expand the infant sample by adding more perinatal individuals.

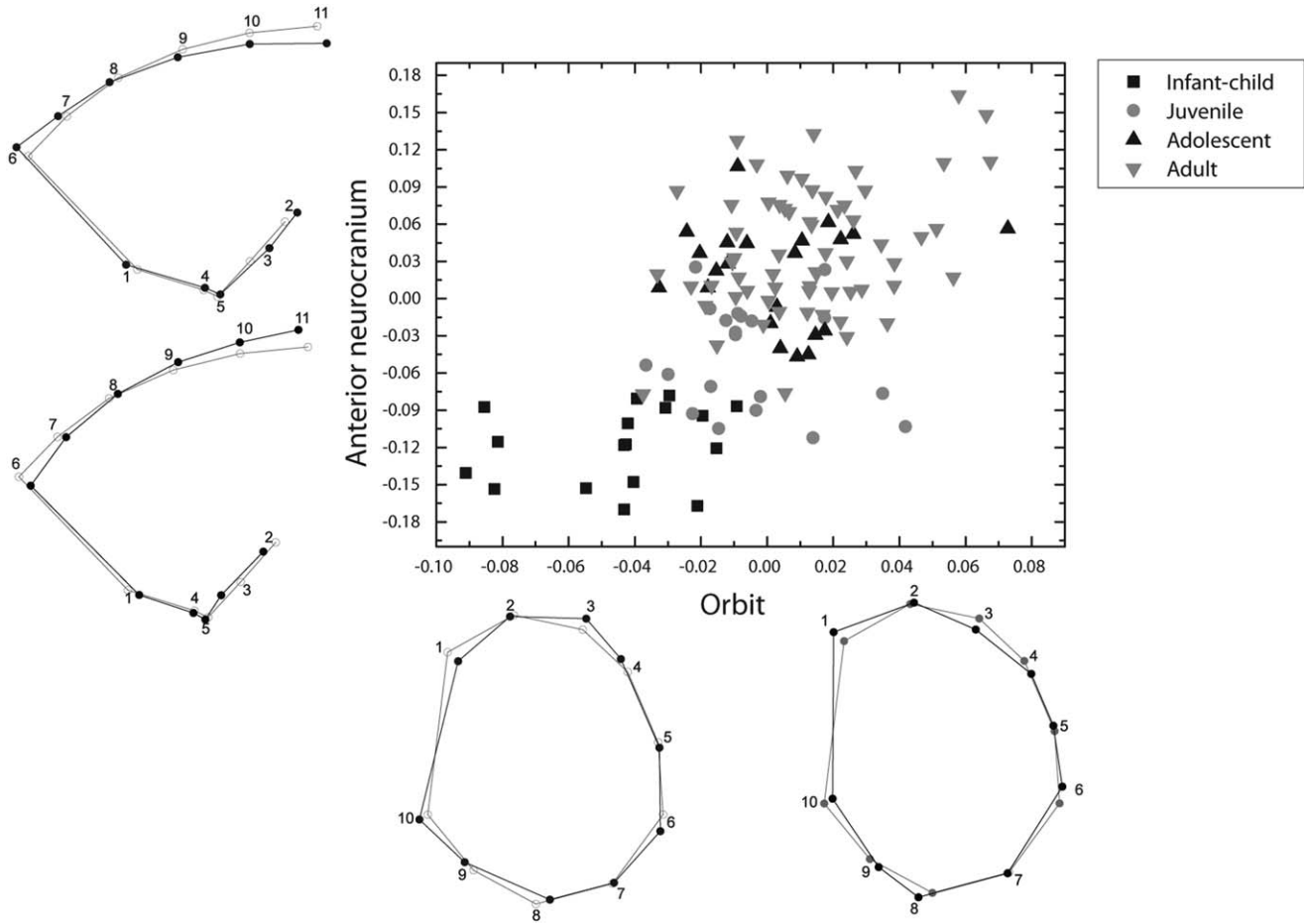


Fig. 5. PLS orbit vs anterior neurocranium. Black wireframes show extreme shape configurations, and grey wireframes represent the mean shape.

TABLE 5. Two-blocks PLS analyses of the orbital rim with the anterior neurocranium and the zygomatic region.

	Singular value PLS1	<i>p</i> -value (perm.)	% total covar. PLS1	Correlation PLS1	<i>p</i> -value (perm.)
Anterior neurocranium Procrustes coordinates PLS	0.00124596	0.0012	74.624	0.58177	<.0001
Anterior neurocranium nlCS-adjusted PLS	0.00089135	0.0389	64.635	0.37264	0.0562
Zygomatic region Procrustes coordinates PLS	0.00079013	0.0078	51.514	0.56917	<.0001
Zygomatic region nlCS-adjusted PLS	0.00047665	0.7131	31.722	0.41427	0.1238

Although most of our results are in line with the assumption that the orbits exhibit a strong relationship with the eyes and neurocranial structures, MB distances indicated that adults are more different in their patterns of covariation, suggesting that there are changes also in later stages of ontogenetic life, even when the neurocranium has reached its maturity. This point gives support to the prediction of facial dynamics influencing orbital morphology, but, according to most of the results, this

expectation cannot be certainly assumed. Forces produced by muscles placed in the temporal fossa are likely to affect orbital morphology. In this work, we found a moderate covariation between orbital aperture and zygomatic shape, suggesting that the masticatory muscles that are anatomically and functionally related to the latter may not markedly affect the development of the orbits. This may be due to the postorbital septum, which protects primate eyeballs from masticatory movements

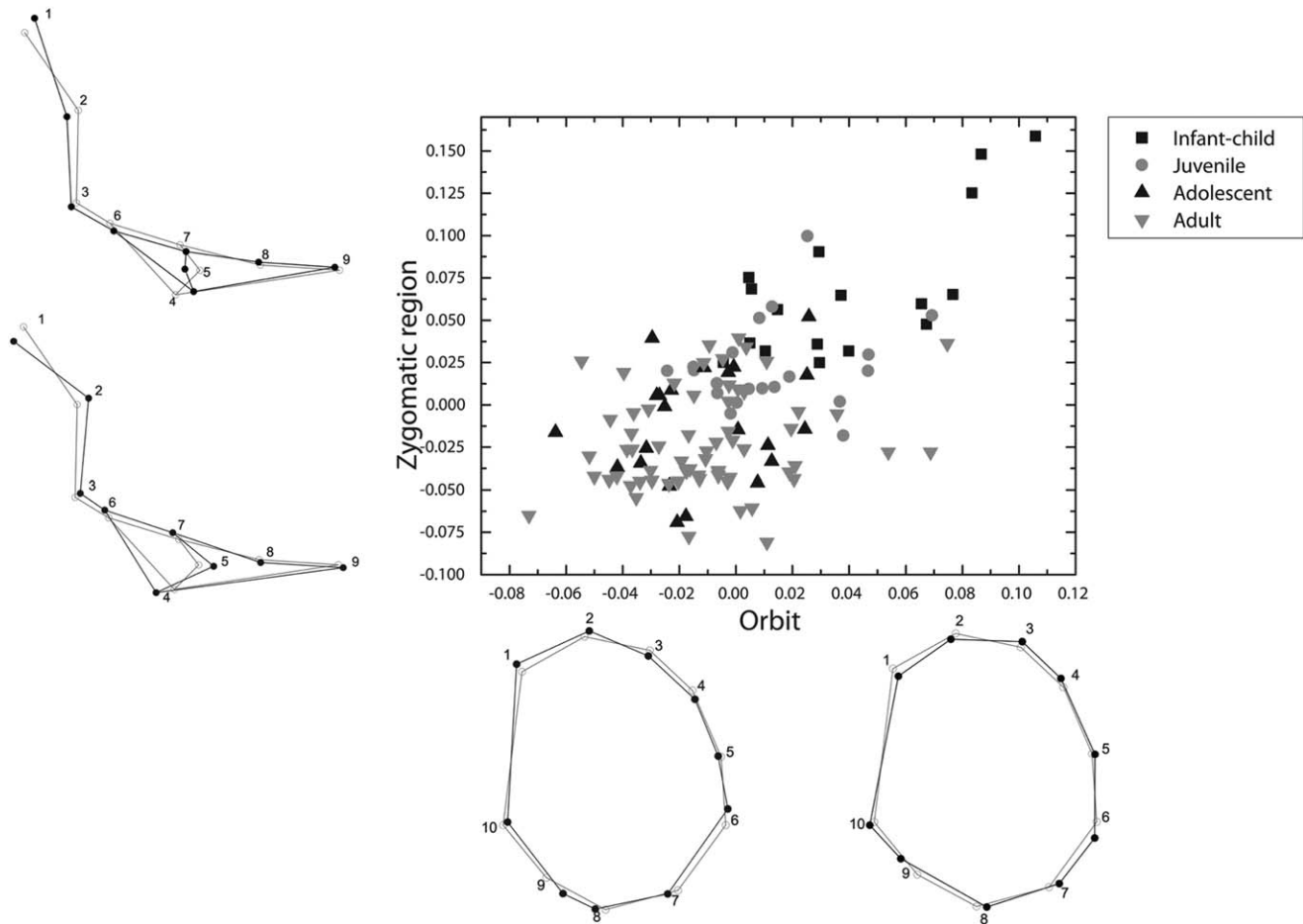


Fig. 6. PLS orbit vs zygomatic region. Black wireframes show extreme shape configurations, and grey wireframes represent the mean shape.

(e.g., Lieberman, 2011). Furthermore, compared to other primates, masticatory muscles are gracile and less developed in humans (Aiello and Dean, 1992; Stedman et al., 2004), and their influence on craniofacial morphology would be minor. On the contrary, forces exerted by the human growing brain are thought to be determinant (Moss and Young, 1960; Cheverud, 1996; Enlow and Hans, 1996). It is worth noting that despite the fact that the effect of sex differences was minimized as described in the Methods section, a bias in the results derived from the unequal proportion of male and female adults cannot be completely rejected. In our study, there are more female individuals in the adult group than males, which could lead to more subtle morphological differences with preceding age groups.

The external orbital rim was investigated by other authors that examined craniofacial differences among populations. Although some populational diversity regarding orbital morphology has been observed (Sardi, 2002; Brown and Maeda, 2004; Sardi et al., 2004, Wu et al., 2007), it has been found to be less variable than other craniofacial traits (Masters, 2008). This is in line with our results: reduced populational variation could be

related to the early establishment of definitive morphological patterns.

While our work does not decipher whether orbital morphology is reliable in phylogenetic studies, it provides some basis for discussion of the processes that are potentially involved in the emergence of morphological variation. The influence of facial structures that develop over a long period of postnatal life and of ecological/environmental factors would be minor, but cannot be completely discarded. If orbits are developmentally linked to the neurocranium, their morphological evolution would also be integrated.

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