

*Original Research Article***Shifts in Subsistence Type and Its Impact on the Human Skull's Morphological Integration**CAROLINA PASCHETTA,¹ SOLEDAD DE AZEVEDO,¹ MARINA GONZÁLEZ,¹ MIRSHA QUINTO-SÁNCHEZ,¹ CELIA CINTAS,¹ HUGO VARELA,² JORGE GÓMEZ-VALDÉS,³ GABRIELA SÁNCHEZ-MEJORADA,³ AND ROLANDO GONZÁLEZ-JOSÉ^{1*}¹Centro Nacional Patagónico, Consejo Nacional de Investigaciones Científicas y Técnicas (CONICET), Puerto Madryn U9120ACF, Argentina²Universidad Nacional de Río Cuarto, Consejo Nacional de Investigaciones Científicas y Técnicas (CONICET), Río Cuarto 5800, Argentina³Departamento de Anatomía, Facultad de Medicina, Universidad Nacional Autónoma de México, México D.F., 04510, México

Objective: Here we evaluate morphological integration patterns and magnitudes in different skull regions to detect if shifts in morphological integration are correlated to the appearance of more processed (softer) diets.

Methods: To do so, three transitional populations were analyzed, including samples from groups that inhabited the same geographical region and for which the evidence shows that major changes occurred in their subsistence mode. Ninety three-dimensional landmarks were digitized on 357 skulls and used as the raw data to develop geometric morphometric analyses. The landmark coordinates were divided into several different regions of biomechanical interest, following a three-level hierarchically nested scheme: the whole skull, further subdivided into neurocranium (divided into the vault and basicranium), the facial (divided into the lower and upper facial), and the masticatory apparatus (divided into alveolar, temporal, and temporo-mandibular joint).

Results: Our results indicate that the morphological integration and variability patterns significantly vary across skull regions but are maintained across the transitions. The alveolar border and the lower facial are the regions manifesting greater value of morphological integration and variability, while the upper facial, the temporo-mandibular joint, and the basicranium are highly integrated and poorly variable.

Conclusions: The transition to softer diets increased morphological variation across cranial regions that are more exposed to masticatory strains effects. *Am. J. Hum. Biol.* 28:118–128, 2016. © 2015 Wiley Periodicals, Inc.

The complex relationship subsumed into the “genotype-phenotype map” concept clearly shows that phenotypic expression is the result of genetic and environmental factors acting jointly (Falconer and MacKay, 1996; Hallgrímsson et al., 2005; Lynch and Walsh, 1998). For instance, the vertebrate’s craniofacial phenotype has a strong genetic determination but skull shape and size variation is also the result of multiple developmental processes affected by varied genetic and epigenetic stimuli (Atchley and Hall, 1991; Enlow, 1990; Hallgrímsson et al., 2002, 2004; Klingenberg et al., 2003; Lieberman, 2002; Wagner, 1996; Wagner and Altenberg, 1996).

Hallgrímsson et al. (2007, 2009) developed the “Palimpsest Model” to explain patterns of phenotypic covariation. The model summarizes the effects of development and environmental factors in the covariation of traits. This model suggests that in the adult skull the pattern of covariation can be seen as the end result of the successive added effects, wherein each of them leaves a distinctive covariance signal corresponding to a developmental process interacting at every developmental phase (Hallgrímsson et al., 2007, 2009). Thus, the model adds all developmental processes generating covariance (e.g., neural crest migration cell, condensation and differentiation cell, muscle–bone interactions, somatic growth) to predict the variation response at any level on the adult morphology.

Consequently, the combined effect of these processes causing the observed covariance pattern may be metaphorically viewed as a medieval palimpsest (a reused scroll on which the shadows of the various texts accumulate over time) (Hallgrímsson et al., 2007, 2009). Among these developmental processes, different muscle–bone interactions, such as the mechanical pressures (e.g., mastication, locomotion) affect key processes in the growth

and development of organisms (Lieberman, 2002), especially at the later developmental phases. Therefore, the study of potential changes in size and shape due to mechanical effects is crucial for understanding phenotypic variability in the skull. Of particular importance are the masticatory loads suffered by the skull in response to variations in stiffness and particle size of diet. Indeed, masticatory loadings have actually had a direct effect on the evolution of the human face (Corruccini, 1999; Corruccini and Handler, 1980; Corruccini et al., 1985; González-José et al., 2005; Ingervall and Bitsanis, 1987; Larsen, 1995, 1997; Lieberman et al., 2004; Lieberman, 2008; Paschetta et al., 2010).

There is a close relationship among the working force of the muscle and bone growth. The mechanical stresses imposed by the muscles induce bone growth, especially before reaching skeletal system maturity (Pearson and Lieberman, 2004). In this context, technological changes associated with the transition from hunter-gatherers to farmers, such as an increase in food processing, have

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TABLE 1. Detail of the studied samples

Economic transition	Latitude	Longitude	Collection	n (F/M/T)	Subsistence	Chronology	Subtotal
Cuyo	35°02'S	68°15'O	Puelches Huarpes	14/14/28 7/5/12	Hunter-Gatherers Farmer	2040–1755 BP 500–800 BP	40
Mexico Central Valley	19°19'N	99°39'O	Tlatelolco/San Gregorio Atlapulco	25/23/48	Farmer	1200–1521 AC	
			San Andrés/San José de los Naturales	71/63/134	Colonial	Century XVII, XVIII y XIX	225
			Actuales	14/29/43	Current	Contemporary	
Ohio River Valley	39°36'N	82°55'O	Indian Knoll Archaic Late Prehistory	40/40/80 01/11/12	Hunter-Gatherers Farmer	10000–3000 BP 1500–500 BP	92
Total				172/185/357			357

F/M/T = Females/Males/Total.

allowed the incorporation of softer diet items, resulting in a reduction of the masticatory activity and a trend toward the gracility of the human skull (Brace et al., 1987, 1991; Carlson and Van Gerven, 1977; Carlson, 1976; Hannam and Wood, 1989; Kiliaridis, 1995; Larsen, 1995, 1997; Lieberman et al., 2004; Pinhasi et al., 2008; Sardi et al., 2006; van Spronsen et al., 1991). Indeed, cooking meat and vegetables changes the fracture toughness of tissues through cellular destruction, either by cellulose softening or collagen denaturation. Experimental studies have shown that cooked vegetables are less rigid, and the stiffness degree being proportional to the cooking time (Lieberman, 2011; Lucas, 2004; Purslow, 1985). Conversely, although meat becomes stiffer when it is cooked, also becomes easier to chew because heat breaks down the collagen fibers causing fracture lines on the tissues (Lieberman, 2011; Lucas, 2004; Purslow, 1985).

The question arises if the transition from a hard to soft diet causes an increase or decrease of the total variance of the skull and its constitutive parts. On one hand, it can be argued that reliance on a harder diet might increase variance as it increases the upper bound of masticatory stress possible while some individuals may use strategies to avoid this stress (such as increasing the number of masticatory cycles) (Peyron et al., 2002, Foster et al., 2006). Harder foods might also produce stresses that are more likely to induce bone remodeling (Capasso et al., 1999; Kennedy, 1989; Weiss, 2010) and transitioning to a softer food might remove this source of covariation. On the other hand, increasing variance as a response to the adoption of softer diets can also be posited as a null hypothesis. The rationale underlying this is that, the “ancestral” or plesiomorphic state of the cranial variation in the human species is constrained by an environment dominated by hard diets. Presumably, the genetic background is intrinsically capable of producing extra phenotypes. However, the masticatory strains would operate via muscle–bone interactions in order to avoid some specific morphospaces, thus decreasing the amount of total population variance. When the environmental determinant disappears, or is relaxed in some way, then the underlying amount of genetic variation is less funneled and potentially able to express phenotypes occupying novel regions in the morphospace (Rutherford, 2000; Waddington, 1957). As there are no previous estimations of variation within transitional cases, we take this second scenario as a point of departure to test some basic hypothesis regarding integration, covariation, and diet type in three different populations experiencing a technological transition. Thus, the aim of this study is to evaluate the variance patterns and morpholog-

ical integration (MI) levels at different regions of the skull to detect whether the increase in variance caused by the appearance of more processed (softer) diets affects integration levels. Specifically, we test the general null hypothesis that there is a linear relationship among integration and variance, no matter the degree of exposure of the different cranial regions and/or subsamples to masticatory stress. This hypothesis can be tested at two levels. First, we evaluate if the emergence of soft diets relaxes the phenotypic canalization imposed by hard diets leading to an increase of phenotypic variation. Second, we also analyze if the cranial regions under strong mechanical masticatory stress show high morphological integration values (relative to variance) due to the high functional demands triggered by masticatory loads.

To test both the hypotheses, we have assembled a sample of three Native American groups that experienced a dramatic change on its subsistence strategy, with little or subtle microevolutionary genetic changes (migration, intrusion, extinction, replacement, etc.). Using such sample, we aimed to maximize environmental differences due to variations in diet, then aiming to isolate the effects of a particular development process: muscle–bone interactions. These interactions are mediated by larger strains on the hard-diet groups, and soft-diet groups are expected to experience less strains and weaker muscle–bone interactions.

MATERIALS AND METHODS

The sample

The sample comprises 357 modern human skulls of both sexes without any visible cranial deformation (Table 1). Sex and age were estimated following diagnostic traits outlined by Buikstra and Ubelaker (1994). On each skull, 90 homologous three-dimensional landmarks coordinates were recorded using a Microscribe G2X digitizer. The muscular attachments, tendons, or cartilage leave marks on the periosteum. The bone remodeling theory suggest that when the muscle attachments are subjected to mechanical stress, the blood pressure increases and stimulates the bone cells formation, resulting in a hypertrophy and an increase in size of the musculo-skeletal marks (Capasso et al., 1999; Jurmain, 1999; Kennedy, 1989; Larsen, 1997; Weiss, 2009, 2010). Thus, besides the classic craniometric landmarks used in the literature, a set of novel landmarks were defined especially to capture the shape of masticatory muscular attachments (Supporting Information Table S1). Muscular attachment marks are useful to define craniometric points, as it is the case

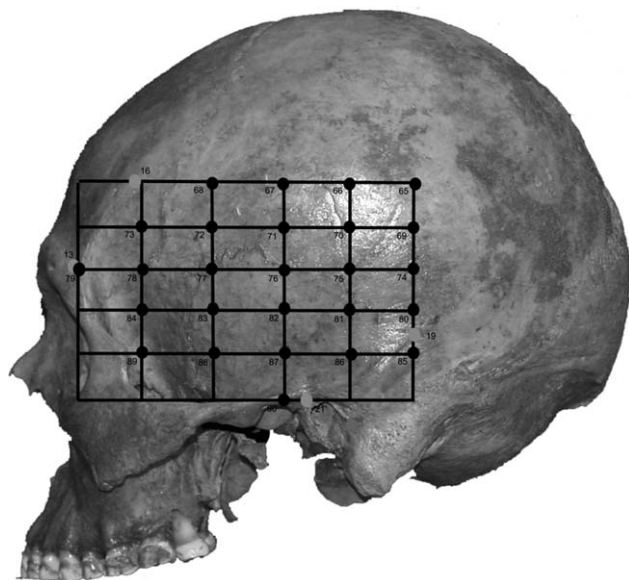


Fig. 1. Landmarks created by gridline intersections on the muscle temporal area. The gray points, 13 (frontotemporal), 16 (stephanion), 19 (enthomion) and 21 (anterior radicular) defined the gridline boundaries. The black points are located on the intersection of vertical and horizontal grid.

regarding the masseter and pterygoid extension. The temporal muscle displays a complex shape due to its semicircular insertion on the vault, which is poorly delimited by anatomical landmarks. To overcome this disadvantage and capture the whole curved surface shape, we used a technique previously applied by Maddux and Franciscus (2009). A grid was projected on the lateral side of the skull using a projector, aimed to cover the area occupied by the temporal muscle. The grid cells were blocked to maintain proportionality when one or both dimensions (horizontal and vertical) are adjusted to the size of the muscle area (Fig. 1). The grid margins are defined by four anatomical points on the boundaries: stephanion (upper point), anterior radicular (lower point), enthomion (posterior point), and frontotemporal (anterior point). The remaining landmark points are then defined by the intersections of the vertical and horizontal grid (Bookstein, 1991; Maddux and Franciscus, 2009; Niewoehner, 2000, 2001, 2005, 2006). From a total of 90 landmark digitized in the whole skull, 64 are craniofacial (craniofacial configuration) and 26 belong exclusively to the temporal area (temporal configuration). As craniofacial and temporal configurations were captured separately, the routine developed by David Reddy (<http://www.nycep.org/nmg/programs.html>) was used to articulate both the configurations.

Cranial regions

The whole configuration of landmarks were divided into hierarchically nested subsets (of “first,” “second,” and “third” order) aiming to recover shape information on regions of different biomechanical implications. It is important to note that to choose these subsets we exclusively used anatomical functional criteria. The hierarchical landmark partitions do not overlap and are mutually exclusive (Supporting Information Table S1). The first hierarchical order corresponds to the entire skull and

comprises all of the 90 digitized points. Three second-order regions were defined: facial, neurocranium, and masticatory. Next, following the classical division of the skull, the neurocranium was further divided into vault and basicranium, representing third-order regions. The facial region was further divided into upper and lower facial (third-order regions), and the masticatory region was further divided into alveolar, temporal, and temporo-mandibular joint (third-order regions).

Subsistence transitions studied

Cuyo. The Argentinean Cuyo area was inhabited by two groups: hunter-gatherer (HG-CU) and farmer (F-CU) populations and were located south and north from the Diamante River, respectively (Cabrera, 1929; Canals Frau, 1937, 1953; Durán, 1994; Latcham, 1929; Michieli, 1978; Prieto, 1989; Sardi et al., 2006). The HG-CU group was assigned to a period of 2040–1755 years BP by radiocarbon dating (Novellino and Guichón, 1999), whereas the F-CU group was assign to the “Late period” (500–800 AP) due to the presence of pottery and irrigation systems (Gambier, 1993).

Mexico Central Valley. These series consist of a transition from precontact farmers (San Gregorio Atlapulco and Tlatelolco) (F-ME), to an early colonial group (San Andrés and San José de los Naturales) (COL-ME), and finally to a contemporary (UNAM-Collection) (Gómez-Valdés et al., 2012), post-colonial group (MOD-ME). For simplicity, and considering that the greatest amount of techniques aimed to preprocess food increased with the contact among Europeans and Amerindians in the sein of the colonial lifestyle, some of the below mentioned tests were performed treating F-ME as the hard-diet group and COL-ME plus MOD-ME as a single soft-diet group. All the specimens belong to sites from the area nearby the City of Mexico.

Ohio River Valley. This collection can be divided into two periods according to their economic strategy. Hunter-gatherers (HG-OH) correspond to the earliest period, which is known as Indian Knoll-Archaic, and expanded from about 10000 years ago BP to 3000 years BP (Cassidy, 1984; Hill, 2003; Jennings, 1974; Winters, 1969). A farmer sample (F-OH) corresponds to a more recent period called Late-Prehistory (1500 to 500 years ago BP; Griffin, 1978; Hill, 2003).

Further comparisons are based on the assumption that masticatory loading decreases with time throughout each regional sample. In other words, we assume that HG-CU, F-ME, and HG-OH represent cases of relative hard-diet environment into each transition case, whereas F-CU, COL-ME, MOD-ME, and F-OH represent their respective softer diet counterparts. There are two issues that should be noted with this assumption. First, while it is true that cooked foods often elicit reduced masticatory loadings, there is nothing to suggest that there is such relationship between cultural tradition and loading. For example, HG populations may not experience the repetitive number of chewing cycles that agriculturalists experience. The total sum of loading events may be higher in agricultural populations even though they may chew relatively less-challenging foods. Second, there are likely numerous other

TABLE 2. ANCOVA results

Model	Df	SS	MS	F-value	P
Cuyo					
TVC	1	3.04 E -07	3.04 E -07	33.956	P < 0.01
Subsistence	1	7.91 E -09	7.91 E -09	0.884	0.359
Interaction (TVC*subsistence)	1	9.86 E -09	9.86 E -09	1.103	0.308
Residuals	18	1.61 E -07	8.94 E -09		
Ohio					
TVC	1	3.64 E -08	3.64 E -08	10.013	P < 0.01
Subsistence	1	1.09 E -09	1.09 E -09	0.299	0.59097
Interaction (TVC*subsistence)	1	3.60 E -10	3.60 E -10	0.1	0.75558
Residuals	18	6.54 E -08	3.63 E -09		
Mexico					
TVC	1	4.98 E -08	4.98 E -08	21.217	P < 0.01
Subsistence	1	2.54 E -09	2.54 E -09	1.082	0.3069
Interaction (TVC*subsistence)	1	1.35 E -08	1.35 E -08	5.742	0.01 < P < 0.05
Residuals	29	6.80 E -08	2.34 E -09		

Significant differences are bolded.

factors that may exacerbate or confound any diet and mastication-related changes. These could include changes in nutrition, medical/dental advances (e.g., in the 20th century sample), lifestyle changes, etc. However, Paschetta (2012) estimated bite forces for the same transitional series used in this work, and found that soft-diet groups present significantly lower bite forces when compared to hard-diet groups. The difference in bite forces (estimated after computing load and lever arms) is observed at the level of cutting bite (at the incisors) and of crushing bite (at the second molars), and this pattern holds for both sexes. Thus, our assumption of greater masticatory loading decreasing with time throughout each regional sample can be preliminarily accepted, at least in the studied samples.

Original landmark configurations were superimposed using Generalized Procrustes Analysis (GPA, Goodall, 1991; Rohlf and Slice, 1990) using the routine implemented in software MorphoJ (Klingenberg, 2011). Superimposition was made independently for each landmark configuration. GPA removes translation, rotation, and scaling effects (Rohlf and Slice, 1990), which enables to conserve independent information on shape and size of the structures under study. All shape information is conserved in the Procrustes coordinates, whereas size information is represented by the centroid size (Dryden and Mardia, 1998). Procrustes superimposition removes any effects of scale but not the allometric shape variation that is related to size or sex. To remove the shape changes due to allometry and sexual dimorphism, we computed a multivariate regression of the Procrustes coordinates on centroid size and sex (Loy et al., 1996; Monteiro, 1999). Since the regression was significant ($P < 0.01$) subsequent statistical analyses were made on the regression residuals and can be considered as allometry-free and sex-corrected data.

Regression of scaled variance of eigenvalues and trace of the variance/covariance matrix. A way to check whether morphological integration (measured as the scaled variance of eigenvalues of the variance/covariance matrix [SVE]) is accompanied by an increase in the phenotypic variance (measured as the trace of the variance/covariance matrix [TVC]) is to perform a regression between these two values. If the structure under study is tightly integrated and also the phenotypic variance is high, a positive, significant regression for both variables is expected (Hallgrímsson et al., 2009).

Traditionally, MI has been measured as the eigenvalues variance of a correlation matrix (Wagner, 1990) or the covariance matrix, depending on the data type (metrics or geometric morphometric data). Each eigenvalue describe the amount of variance associated to their corresponding eigenvector. When a given correlation/covariance matrix can be decomposed into a few first eigenvalues that are quite large in comparison to the rest of the eigenvalues, the trait is considered to be highly integrated because the variation of the traits involved is confined to a small subspace in the overall multivariate phenotypic space (Wagner, 1990; Willmore et al., 2006). Based on this criterion, Pavlicev et al. (2009) suggested the relative eigenvalue variance or the SVE as an estimation of the magnitude of covariance independent of the number of features; therefore, it can be seen as a useful parameter to compare morphological integration among variance/covariance matrix of different dimensions. To evaluate if morphological integration (measured as the SVE) is accompanied by an increase in the phenotypic variance, we regressed SVE on the TVC computed for each cranial region and subsample. Confidence intervals for SVE and TVC were obtained resampling each dataset with replacement for 1000 iterations from each variance-covariance matrix of Procrustes coordinates. The resampling dataset were fixed to represent the lowest sample size in the overall sample in order to avoid bias on the computation of TVE and SVE range due to the unequal subsamples sizes (following Hünemeier et al., 2013).

Theoretically, as subsistence shifts can differ regarding their effects on cranial shape, we also tested for differences among the regression (SVE on TVC) lines of hard versus soft diet, within each transition case. To do so, we computed an analysis of covariance (ANCOVA) test for each transition case (Table 2).

Finally, a series of *t*-tests were performed in order to check for hard diet versus soft diet differences in the SVE and TVC for the different cranial structures and transitions. Note that in this case, the Mexican farmer group (F-ME) was considered as the hard-diet group, whereas the pooled colonial (COL-ME) and modern (MOD-ME) series were considered as a single, soft-diet group (see above).

RESULTS

Prior to exploring the relationship among morphological integration degree and its association with the amount

of variance within each cranial region we first evaluated if the regression of the SVE on the TVC differed among hard versus soft-diet groups within each subsistence transition studied here. The ANCOVA results (Table 2) show that the regression of SVE on TVC is nonsignificantly different between hard versus soft-diet groups in the case of Cuyo (Fig. 2) and Ohio (Fig. 4). The Mexican case results on significantly different regression lines. Thus, in addition

to discussing this new result, we discuss the relationship among SVE and TVC on a separate way (hard diet versus soft diet) in the Mexican case.

The regression of SVE on TVC for the Cuyo transition (Fig. 2) was significant ($r = 0.79, p = 0.00001$). Some specific cranial regions behave as more integrated than expected. For instance, the basicranium (ba), the upper facial (fupp), and the temporo-mandibular joint (tmj) displayed a larger than expected degree of morphological integration estimator (SVE) relative to their total variance estimator (TVC). The remaining skull regions fall within the confidence limits of the regression line or below it. The alveolar (alv) and the lower facial (flow) of agriculturalist tend to show large values for both, TVC and SVE, and to a lesser degree, the alveolar (alv) and the lower facial (flow) of hunter-gatherer.

Since the ANCOVA results (Table 2) indicated significantly different slopes for the hard diet versus soft diet regressions of SVE on TVC to Mexico, we analyzed these slopes on an independently (Fig. 3a, b). For the hard-diet group (Fig. 3a) the regression line was significant ($r = 0.6572, p = 0.001508$). The temporo-mandibular joint (tmj) behaves as in the previous case: it displays greater than expected integration estimator (SVE) relative to their TVC. The upper facial (fupp) and basicranium (ba) show large values for both estimators, whereas the alveolar (alv), lower facial (flow) and the facial (fa) display intermediate values of variation (TVC) and low values of integration (SVE). The remaining skull regions are within the confidence limits of the regression line. The soft-diet group formed by the colonial and modern series pooled together (Fig. 3b) presented a significant regression of SVE on TVC ($r = 0.2993, p = 0.004952$), where the temporo-mandibular joint (tmj), the basicranium (ba), and the upper facial (fupp) showed higher values of SVE (integration) relative to its TVC value (to the exception of the basicranium in the modern group).

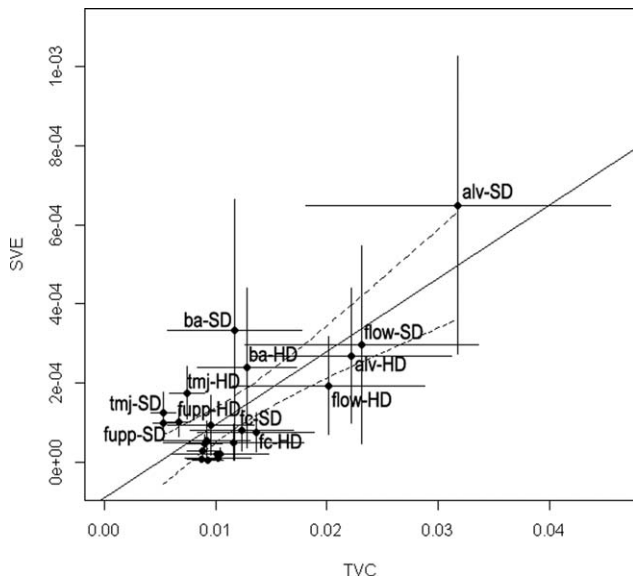


Fig. 2. Regression of SVE on TVC for each cranial region and economies of Cuyo. Error bars are standard deviations obtained by resampling the original data with replacement. Ba, basicranium; tmj, temporo-mandibular joint; fupp, upper facial; alv, alveolar; flow, lower facial; fc, facial; HD, hard diet; SD, soft diet.

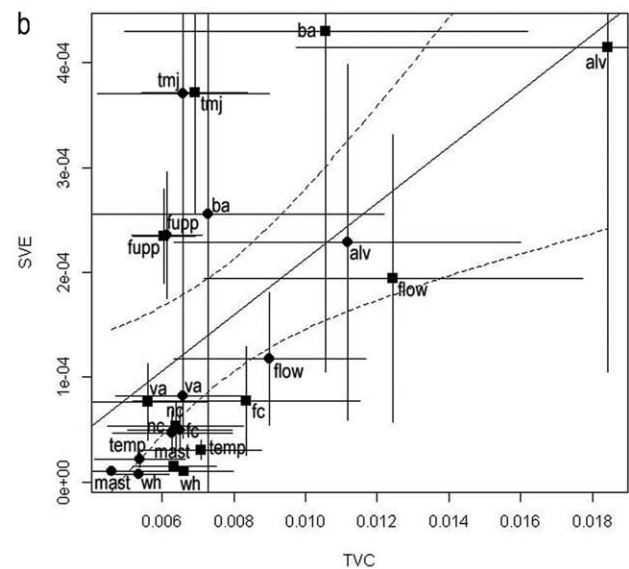
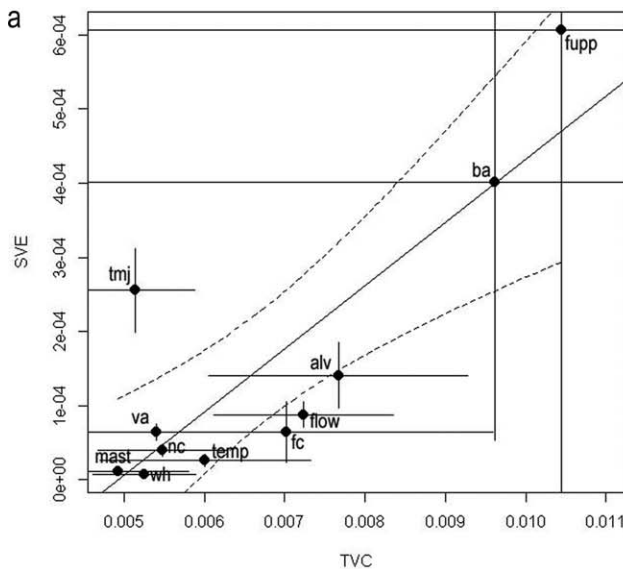


Fig. 3. Regression of SVE on TVC for each cranial region of Mexico. (a) Hard-diet group. (b) Soft-diet group (square = modern group, circle = colonial group). Error bars are standard deviations obtained by resampling the original data with replacement. ba, basicranium; tmj, temporo-mandibular joint; fupp, upper facial; alv, alveolar; flow, lower facial; fc, facial; va, vault; mast, masticatory; nc, neurocranium; wh, whole skull; temp, temporal; HD, hard diet; SD, soft diet.

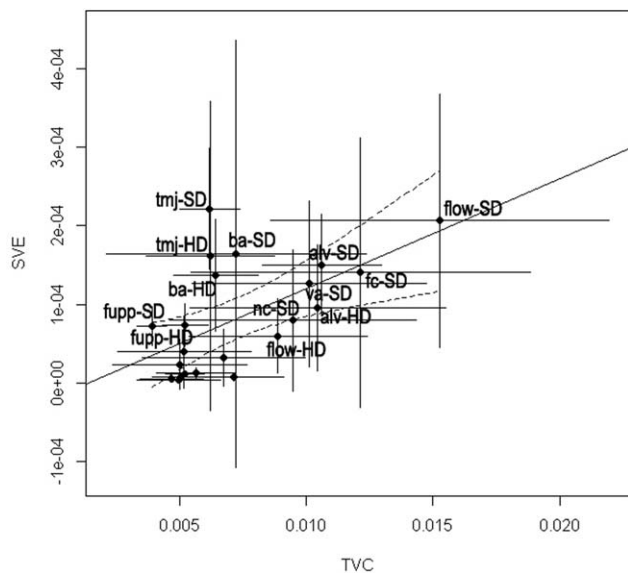


Fig. 4. Regression of SVE on TVC for each cranial region and economies of Ohio. Error bars are standard deviations obtained by resampling the original data with replacement. ba, basicranium; tmj, temporo-mandibular joint; fupp, upper facial; alv, alveolar; flow, lower facial; va, vault; nc, neurocranium; HD, hard diet; SD, soft diet.

The regression obtained on the Ohio series is significant ($r = 0.68$, $p = 0.0005$) but the behavior of the cranial regions slightly differs from the above transitions (Fig. 4). The lower facial (flow) and alveolar (alv) regions on the hard-diet group present the highest values of TVC, but below the confidence limits, thus present lower integration than expected. In this transition, the upper facial (fupp), the temporo-mandibular joint (tmj), and the basicranium (ba) are regions presenting SVE scores well above the expected values, or within them but close to the upper limits.

As a whole, our results suggest that our null hypothesis of a linear association among integration and variation across transitions and cranial regions cannot be rejected in general, but that a more detailed inspection of results indicates some regular deviations from the general expectation. There is a general pattern of integration and variation throughout the three transitional analyses (Figs. 2–4). In one hand, the general results indicate that, the upper facial (fupp), temporo-mandibular joint (tmj), and basicranium (ba) tend to present a value of morphological integration (SVE) higher than expected for the values of variance (TVC). This pattern suggests a strong canalization and a relative stasis across different Native American populations that diverged during the settlement of the New World and its subsequent admixture with Europeans (in the case of Mexicans). On the other hand, the alveolar (alv) and the lower facial (flow) generally show high values for both the variables (SVE and TVC), meaning that these cranial structures are strongly integrated and there are also highly variable.

To further evaluate if the above suggested overall relationship between SVE and TVC and their recurrent deviations is not influenced by the small sample size of the groups, we plotted the entire resampled data corresponding to the whole skull (see Fig. 5) and cranial regions

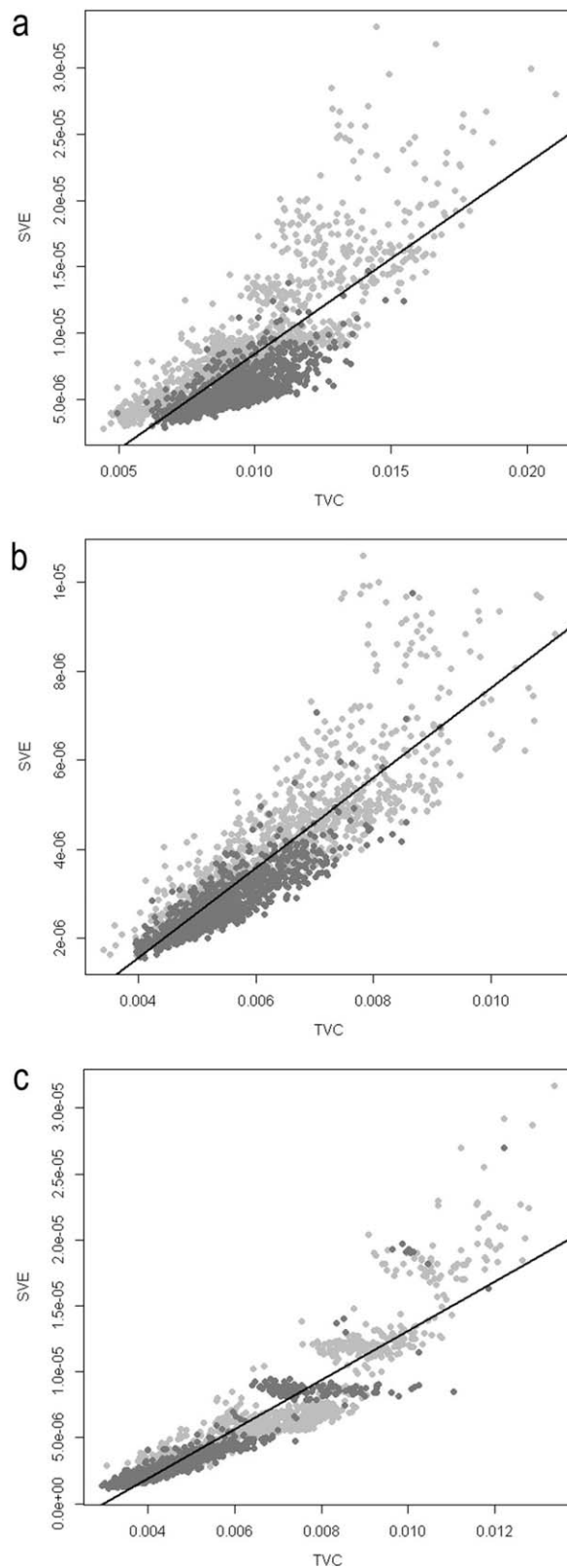


Fig. 5. The relationship between variance and integratedness in the three populations studied. Plot of the resampled data for (a) Cuyo, (b) Mexico, and (c) Ohio. Dark gray = hard-diet group; light gray = soft-diet group.

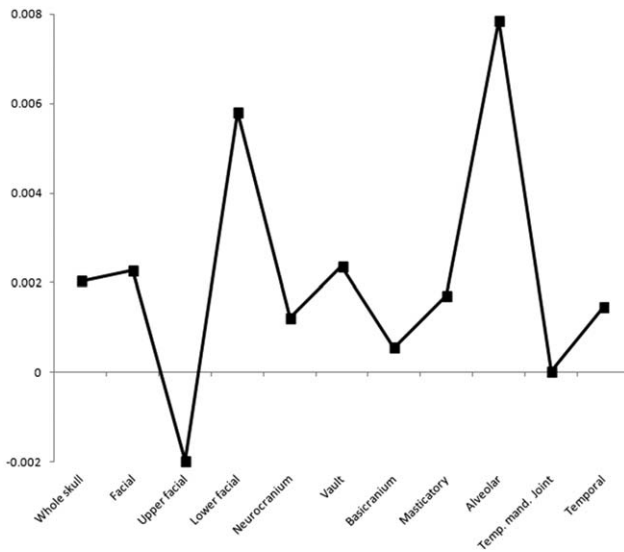


Fig. 6. TVC values obtained from the difference between the mean of TVC of soft diets and the mean of TVC of hard diets for different regions of the skull. Positive values indicate that the mean of TVC of soft diet is higher than the mean TVC of hard diets.

within each transition and diet type (see Supporting Information Figs. S1–S3). This is a graphical solution introduced by Hallgrímsson et al. (2009) to work around the problem of differing sample sizes. The plot of the resampled data confirms the trends observed in the regression results. For instance, the behavior of some regions presenting above the expected integration, such as the basicranium, the temporo-mandibular joint, and the superior face (orange, black, and yellow points in Supporting Information Figs. S1–S3) represent a ubiquitous and conserved pattern observed across the three transitions and regardless of the diet hardness. Moreover, it is evident from Figure 5 that softer diet groups occupy the extreme values of the TVC axis, thus exhibiting higher variance levels in comparison to hard-diet groups (see below). Finally, these plots indicate that even though there are correlations between SVC and TVC within the resampled distributions for each subsistence, the overall trend in the data is due to the among-subsistence correlation between integration and variance.

Noticeably, the TVC values of the processed or soft-diet groups tend to be greater than the hard-diet counterpart to the same structure and in the three transitions. Figure 6 shows the difference between the mean TVC of the soft and hard-diet groups for each region of the skull. This graph indicates that only on the upper facial (fupp) area, the hard-diet groups have greater variance than in the soft-diet group. Remarkably, the remaining structures exhibit higher TVC values on the soft-diet groups. The two regions with the biggest hard diet versus soft diet differences are the alveolar (alv) and the lower facial (flow), a pattern which is also evident in Figures 2–4. The lowest variances are located at the upper facial (fupp), the basicranium (ba) and temporo-mandibular joint (tmj), which are precisely those which are, in general, above the regression lines.

Table 3 shows the result of the t-test between hard and soft-diet groups aimed to detect differences in SVE and

TVC for each skull region and transition. The results indicate that most regions are significantly different in terms of SVE and TVC. Although the main objective of our article is focused on the relationship among SVE and TVC, this result supports the fact that the differences among hard and soft-diet groups have different expressions including shape, size, integration, and variance changes.

DISCUSSION

Masticatory strains are among the most important environmental influences that modern human populations experienced during its recent evolution (Corruccini and Handler, 1980; Corruccini et al., 1985; Corruccini, 1999; González-José et al., 2005; Ingervall and Bitsanis, 1987; Larsen, 1995, 1997; Lieberman et al., 2004; Lieberman, 2008; Paschetta et al., 2010; Paschetta, 2012). This is so because technological innovation enabled the acquisition of new food items, and a vast array of procedures and preparation skills that, as a whole, contribute to a general softening of diet. Cooked food (meat and vegetables) acquires properties that make it softer and easily digestible in front of raw food. Experimental studies have shown that cooked vegetables are less rigid, whereas meat becomes stiffer, making it easier to chew because heat breaks down collagen causing fracture lines on the tissues (Lieberman, 2011; Lucas, 2004; Purslow, 1985). Even when there is no straightforward relationship among masticatory loadings and technological advances on food preparation, we have previously demonstrated that bite forces decrease across transitions, thus suggesting that economic transition is accompanied by softer diets, less chewing cycles, etc. (Paschetta, 2012).

Traditionally, the impact of technological/subsistence transitions has been approached on the basis of raw size or shape changes. In other words, the focus has been to test experimentally on model animals (Herring and Teng, 2000; Hylander and Johnson, 1992; Hylander et al., 1991; Lieberman et al., 2004; Ravosa et al., 2000; Ross and Hylander, 1996; Ross, 2001) or comparatively on human natural populations (Carlson and Van Gerven, 1977; Carlson, 1976; Corruccini and Handler, 1980; González-José et al., 2005; Hinton and Carlson, 1979; Larsen, 1995, 1997; Lieberman, 1996; Paschetta et al., 2010; Sardi et al., 2006) if the exposition to softer diets triggers plastic changes in the skull during the final phases of the developmental trajectory towards the adult form. In humans, experimental approaches are difficult to carry on, and only one quantified shifts in facial growth in response to mechanical stress (Ingervall and Bitsanis, 1987). The authors examined the effect of chew a chewing gum for two hours daily for one year in children (7–12 years old). The volunteers that underwent the experiment developed greater masticatory forces and a larger size of their jaws and maxillary arches. The majority of studies performed in humans were developed on natural human populations which have experienced changes in their economic strategies. Overall, these studies agree about the notion that the impact of hard-food consumption is an increase in the overall robustness (Larsen, 1995, 1997), or size (Sardi et al., 2006) of the skull, an increase of facial region relative to whole skull (Carlson and Van Gerven, 1977), the increase of temporal muscle area (Carlson and Van Gerven, 1977; Carlson, 1976; González-José et al., 2005; Sardi et al., 2006), also an increase of temporo-

TABLE 3. Comparison of SVE and TVC scores between hard and soft diet groups

	Cuyo			Mexico			Ohio		
	Mean HD	Mean SD	t-Value P	Mean HD	Mean SD	t-Value P	Mean HD	Mean SD	t-Value P
Whole skull	0.009273	0.010232	20.34 0.18 0.01 < P < 0.05	0.0052983	0.0052983	13.31 5.48 0.01 < P < 0.05	0.004974	0.007130	5.61 2.95 0.11
Neurocranium	0.008863	0.009188	55.66 0.21 0.01 < P < 0.05	0.0054264	0.0058367	20.90 7.79 0.01 < P < 0.05	0.000004	0.000010	3.57 2.28 0.26
Basicranium	0.012814	0.011689	21.78 0.11 0.01 < P < 0.05	0.0094782	0.0084465	10.14 6.25 0.01 < P < 0.05	0.007234	0.006440	17.17 10.99 0.01 < P < 0.05
Vault	0.008986	0.009580	31.28 0.20 0.01 < P < 0.05	0.0053518	0.0059825	15.24 1.93 0.01 < P < 0.05	0.005189	0.010120	3.10 1.93 0.30
Facial	0.000047	0.000093	30.51 0.05 0.01 < P < 0.05	0.0000176	0.0000198	13.33 4.57 0.01 < P < 0.05	0.000040	0.000130	4.64 2.00 0.14
Lower facial	0.000075	0.000081	29.24 0.10 0.01 < P < 0.05	0.0000182	0.0000140	4.57 6.42 0.01 < P < 0.05	0.000032	0.000100	2.00 4.27 0.29
Upper facial	0.020157	0.022153	21.20 0.07 0.01 < P < 0.05	0.0072039	0.0080793	6.42 2.80 0.01 < P < 0.05	0.009474	0.015270	2.27 4.27 0.15
Masticatory	0.000193	0.000269	77.91 0.25 P < 0.01	0.000189	0.000227	4.88 1.67 0.01 < P < 0.05	0.000080	0.000210	2.27 6.91 0.09
Alveolar	0.006728	0.005339	8.68 0.05 P < 0.01	0.0107675	0.0084179	4.88 9.88 0.01 < P < 0.05	0.005234	0.003910	6.91 184.81 P < 0.01
Temporo-mandibular joint	0.000100	0.000097	11.69 0.25 0.01 < P < 0.05	0.0003090	0.0001819	9.88 6.59 0.01 < P < 0.05	0.000074	0.000070	25.72 5.88 0.11
Temporal	0.008752	0.010389	11.69 0.10 0.01 < P < 0.05	0.0049362	0.0047647	3.71 2.35 0.01 < P < 0.05	0.004692	0.005070	14.88 28.98 0.01 < P < 0.05
	0.000008	0.000020	2.44 0.11 0.01 < P < 0.05	0.0000033	0.0000028	11.01 5.77 0.01 < P < 0.05	0.000005	0.000010	5.88 14.88 P < 0.01
	0.023142	0.031774	6.36 0.11 0.01 < P < 0.05	0.0075661	0.0093043	3.71 2.35 0.01 < P < 0.05	0.012150	0.010620	14.88 28.98 0.01 < P < 0.05
	0.000297	0.000649	2.68 0.25 0.01 < P < 0.05	0.0000328	0.0000478	11.01 5.77 0.01 < P < 0.05	0.000140	0.000150	28.98 642.51 P < 0.01
	0.007478	0.005318	5.92 0.11 0.01 < P < 0.05	0.0051257	0.0058627	11.01 5.77 0.01 < P < 0.05	0.006216	0.006200	642.51 6.41 P < 0.01
	0.000175	0.000125	6.00 0.25 0.01 < P < 0.05	0.0000503	0.0000710	11.66 7.33 0.01 < P < 0.05	0.000162	0.000220	6.41 25.35 0.01 < P < 0.05
	0.010087	0.011595	14.38 0.25 0.01 < P < 0.05	0.0059889	0.0056718	11.66 7.33 0.01 < P < 0.05	0.005639	0.005210	25.35 21.98 0.01 < P < 0.05
	0.000021	0.000050	2.39 0.25 0.01 < P < 0.05	0.0000097	0.0000082	7.33 0.25 0.01 < P < 0.05	0.000013	0.000010	21.98 0.000010 0.01 < P < 0.05

Significant differences are bolded.

mandibular joint (Corruccini and Handler, 1980; Hinton and Carlson, 1979; Paschetta et al., 2010; Paschetta, 2012), and an increase of thickness of the cranial vault bones (Lieberman, 1996).

Despite the aforementioned effort on testing for raw size and shape differences among hard and soft-diet groups, it remains to be discussed if variation in the masticatory strains also affects the level of morphological integration among traits. The relation between morphological integration and variability is based on the relative independence between structures (Wagner and Altenberg, 1996; Willmore et al., 2007). A strong integration between features constrains the variability because a change in a tightly integrated feature rarely is advantageous for other structure, or the organism. Therefore, changes in individual parts are likely to be selected against (Wagner and Altenberg, 1996; Willmore et al., 2007).

The link among masticatory strains and morphological integration is interesting, since muscle-bone interactions affected by variations in masticatory strains due to technological innovation can be seen as a developmental process that generates covariance patterns during the final phases of the bone modeling and remodeling that ends up with the adult skull in vertebrates. In fact, epigenetic interactions between muscle and bone in the skull are frequently cited as a cause of covariation among skeletal elements (Cheverud, 1982; Hallgrímsson et al., 2004, Willmore et al., 2006), and bone growth has been demonstrated to be affected by the mechanical effects of muscle activity (Herring, 1993). Even when mechanical influence on bone growth can be detected during the prenatal phases of development (Delaere and Dhem, 1999; Hall and Herring, 1990; Herring and Lakars, 1982), their influences are clearly more notorious after solid food begins to represent a mandatory item in the diet and mastication mechanics begin. As a consequence, osseous structures operating as muscle attachments or as receptors of mastication loads will share common epigenetic influences that are produced by muscle activity (Hallgrímsson et al., 2007). Such effects would produce covariation among structures that share influences (e.g., by muscles such as the masseter and the temporal) or are similarly affected by the dispersal of mechanical forces during mastication (Lieberman et al., 2004; Zelditch et al., 2006; Hallgrímsson et al., 2007).

Thus, we have taken the Palimpsest model (Hallgrímsson et al., 2007, 2009) as a theoretical framework to measure differences in the covariance structure across samples differing mainly on its degree of exposure to mechanical loadings and masticatory strains, in order to infer which patterns of covariance are determined by a particular developmental process such as muscle-bone interactions.

As mentioned above, two potential scenarios can be stated to predict if variance will increase or decrease as a response to the adoption of softer diets. Our results, specifically the comparison of TVC values presented in Figures 5 and 6, suggest that soft-diet groups display a greater amount of variance when compared to their hard-diet counterparts. Hallgrímsson et al. (2009) argued that the increase in the variance does not necessarily means an increase in morphological

integration. Their experimental results on knock-out mice, largely inspired by those obtained by Waddington (1942) show that a mutation in an individual have a significant phenotypic effect, generally resulting in a variance increase as a consequence of change in the population average. Therefore, Hallgrímsson et al. (2009) suggest that the estimation of phenotypic integration cannot be disconnected from the notion of variance, and that intentional mutations result in an increased of variability because of the appearance of some phenotypic variants that were not present before mutation. Extrapolating this reasoning to the particular case of subsistence-type transitions, the acquisition of soft, more processed diets can be seen as the source of novel variation used to contrast the levels of integration. The increasing in variation at the soft-diet groups is evident in Figure 6 for all skull regions excepting the upper facial.

The lower facial region is one of the most affected by hardness of diet: its response to changes in diet characteristics is evident on the size, shape, morphological integration degree and phenotypic variation (Paschetta, 2012). This evidence supports the suggestion by several authors that the inferior part of the face is strongly affected by diet properties (Hylander and Johnson, 1992; Hylander et al., 1991; Lieberman, 2011; Ross and Hylander, 1996; Ross, 2001). Paradoxically, the alveolar region present some changes in the pattern of morphological integration, although less evident than those observed in the lower facial.

Besides some particularities observed in the Ohio transition (high TVC and SVE values to neurocranium, vault and facial of farmers), a remarkable result is that the regression pattern is very similar across transitions. This indicates that some structures such as the cranial base, the temporo-mandibular joint, and the superior part of the face are highly integrated, beyond the genetic differences among them and the geographic environment in which they developed. Furthermore, these results indicate a high level of genetic canalization that remains constant under environmental fluctuations. Strong canalization was also observed in mutant mice displaying Apert Syndrome that share the general pattern of morphological integration with their non-mutant littermates (Martínez-Abadías et al., 2011). Also, studies on phenotypic covariance structures of Neotropical primate skulls demonstrated that it have remained relatively constant, even across phyletic diversification that enabled the phenotypic means to evolve during the last 30 million years (Marriog and Cheverud, 2001). A similar result was obtained in human populations, where covariance has also been shown to be stable (González-José et al., 2004, Hünemeier et al., 2012), even under extreme mechanical stressors experienced during early postnatal phases, as is the case of intentionally deformed skulls (Martínez-Abadías et al., 2009).

Our results support the abovementioned examples of stasis in some integration patterns across different genetic and environmental landscapes, which in conjunct indicate that a strong phenotypic canalization operates during the cranial evolution of primates in general and humans in particular. However, our results bring additional insights about which specific structures remain strongly integrated in face of an increasing of variation due to changes in muscle–bone interaction patterns. From the Palimpsest model point of view (Hallgrímsson et al.,

2007, 2009), our results are valuable since they arise from considering the relationship among total variance and covariation and from focusing on a sample maximizing differences on a specific developmental process without requiring an experimental approach.

CONCLUSIONS

The transition to softer, more processed diets increased the variation of the cranial regions more exposed to the effects of masticatory strains. In other words, specimens belonging to soft-diet groups tend to occupy new regions of the morphospace, maybe as a consequence of relaxing of the functional demands imposed by chewing hard food. The evolution toward soft diets, then unveils cryptic phenotypic variation.

The pattern of morphological integration and variation differs across cranial regions, being the lower facial and the alveolar regions the ones displaying greater variance and integration. In contrast, regions not directly involved in the masticatory mechanics tend to present above than-average integration, suggesting greater canalization as a response to variations in masticatory loads.

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