

# The Influence of Masticatory Loading on Craniofacial Morphology: A Test Case Across Technological Transitions in the Ohio Valley

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**ABSTRACT** Masticatory loading is one of the main environmental stimuli that generate craniofacial variation among recent humans. Experimental studies on a wide variety of mammals, including those with retrognathic postcanine teeth, predict that responses to masticatory loading will be greater in the occlusal plane, the inferior rostrum, and regions associated with the attachments of the temporalis and masseter muscles. Here we test these experimentally-derived predictions on an extinct human population from the middle and upper Ohio valley that underwent a marked shift from hunting-gathering to extensive farming during the last 3,000 years and for which we have good archaeological evidence about diet

and food processing technology. Geometric morphometric methods were used to detect and measure the putative effect of diet changes on cranial shape independent of size. Our results partially confirm only some of the experimental predictions. The effect of softer and/or less tough diets on craniofacial shape seem to be concentrated in the relative reduction of the temporal fossa and in a displacement of the attachment of the temporal muscle. However, there were few differences in craniofacial shape in regions closer to the occlusal plane. These results highlight the utility of exploring specific localized morphological shifts using a hierarchical model of craniofacial integration. *Am J Phys Anthropol* 141:297–314, 2010. © 2009 Wiley-Liss, Inc.

The adult form of complex phenotypes, such as craniofacial size and shape, derives from multiple, intricate developmental processes that are affected by both genetic and epigenetic stimuli (Cheverud, 1988; Enlow, 1990; Atchley and Hall, 1991; Wagner, 1996; Wagner and Altenberg, 1996; Hallgrímsson et al., 2002, 2004; Lieberman et al., 2002; Klingenberg et al., 2003; González-José et al., 2004). Even though there is evidence supporting genetic determinism on craniofacial form (Martínez-Abadías et al., 2009), there is a general appreciation that epigenetic stimuli play a major role in influencing overall skull shape both from interactions within the organism (e.g., patterning of migrating neural crest cells as they come into contact with mesoderm) as well as from environmental stimuli (see Beecher and Corruccini, 1981; Hallgrímsson et al., 2007a,b). Among the many environmental factors that may have influenced the evolution of the highly derived human face, masticatory loading in response to variations in hardness, toughness, and particle size in diet is thought to be particularly important (Corruccini and Handler, 1980; Beecher and Corruccini, 1981; Corruccini and Beecher, 1982, 1984; Beecher et al., 1983; Corruccini et al., 1985; Ingervall and Bitsanis, 1987; Larsen, 1995, 1997; Ciochon et al., 1997; Corruccini, 1999; Lieberman et al., 2004; González-José et al., 2005; Lieberman, 2008). Previous studies agree that hard, tough and/or unprocessed diets generally lead to an increase in the overall robusticity (Larsen, 1995, 1997), or size (Sardi et al., 2006) of the skull, an increase in facial size relative to total size

(Carlson and Van Gerven, 1977), an increase in temporal muscle area (Carlson, 1976; Carlson and Van Gerven, 1977; González-José et al., 2005; Sardi et al., 2006), an increase in temporo-mandibular joint size (Hinton and Carlson, 1979; Corruccini and Handler, 1980) and an increase in the cranial vault thickness (Hylander, 1986; Lieberman, 1996).

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Since hard nonprocessed diets are the ancestral condition prior to the invention of cooking and other food processing technologies, some degree of craniofacial change is expected to result from technological changes over the last few thousand of years that have permitted humans to eat softer, less tough, and more processed foods. In particular, it is frequently assumed that a reduction in masticatory muscle activity and a concomitant decrease in mechanical loading of the craniofacial skeleton are responsible for a reduction in muscle size and their related structures. Further, because mechanical strains induce bone growth, especially prior to skeletal maturity (for review, see Pearson and Lieberman, 2004), and because chewing harder and tougher foods generates higher strains in the lower face (see below), less processed diets should lead to relatively more craniofacial growth in the mandible, lower maxilla, and other regions subject to greater loading (for review, see Wood and Lieberman, 2001). In this context, technological changes related to the shift from hunting-gathering or foraging to food production and cooking, as well as the incorporation of softer foods are hypothesized to have led to reduced masticatory activity and the concomitant gracilization trend observed among recent humans (Carlson, 1976; Carlson and Van Gerven, 1977; Brace et al., 1987; Hannam and Wood, 1989; Brace et al., 1991; van Spronsen et al., 1991; Kiliaridis, 1995; Larsen, 1995, 1997; Lieberman et al., 2004; Sardi et al., 2006; Pinhasi et al., 2008).

Although the importance of mechanical loading on facial growth is well appreciated, how the face is loaded during mastication is only partially understood, especially in the human face, which is highly derived relative to other primates in several respects such as the presence of a retrognathic face, and a reorientation and expansion of the surface of the face in the coronal plane (Lieberman et al., 2004). Nevertheless, experiments on a wide variety of nonhuman primates and other mammals permit several reliable inferences. First, mastication generates a gradient of strains in the face with highest strains experienced near the occlusal plane, moderate strains in the middle face, and very low strains in the upper face (e.g., Hylander et al., 1991; Hylander and Johnson, 1992; Ross and Hylander, 1996; Ross, 2001; Lieberman et al., 2004). Second, regions of muscle attachment and insertion such as the zygomatic arch and the coronoid process probably experience locally high strains (e.g., Hylander et al., 1991; Hylander and Johnson, 1992; Lieberman et al., 2004). Finally, during unilateral mastication, the retrognathic face is likely twisted in the coronal plane and both bent and sheared in the sagittal plane (Ross and Hylander, 1996; Herring and Teng, 2000; Ravosa et al., 2000; Lieberman et al., 2004). Many experimental studies on nonhuman mammals have shown that highly processed diets lead to dramatically lower rates of craniofacial growth in the lower face leading to malocclusions and dysmorphologies (Corruccini and Beecher, 1982, 1984; Beecher et al., 1983; Corruccini, 1999). Most of these studies, however, compared growth in animals fed laboratory foods such as chow with softened ground-up chow. In one of the experimental studies conducted so far on the effects of cooking more normal foods (Lieberman et al., 2004) hyraxes fed microwaved carrots, apples, potatoes, kales and rabbit chow had significantly less growth in the ventral and posterior portions of the face, where strains are highest.

One of the advantages of experimental studies is the ability to control for the effect of alternative sources of environmental and genetic variation that clearly play an important role in natural populations. However, natural populations are subject to an unknown number of different environmental stimuli which act simultaneously and in an integrated fashion on cranial growth and development. For instance, among most hunter-gatherers hardness and toughness of the diet is usually accompanied by varying levels of abrasiveness. Moreover, parafunctional dental use is ubiquitous among hunter-gatherer populations. Obviously, sex and age contribute further sources of uncontrolled variation. In summary, classifying diets as "hard" versus "soft" or "processed" versus "unprocessed" in natural human populations oversimplifies a complex array of masticatory stimuli influenced by many parameters such as hardness, toughness, particle size, as well as other factors such as age of weaning, abrasives in the diet, paramasticatory loading, and so on. In summary, there is a strong need to complement experimental studies of the effects of masticatory loading with comparative analyses of natural populations.

There have been several previous comparative studies of craniofacial shape in populations that ate different diets (Carlson, 1976; Carlson and Van Gerven, 1977; Hinton and Carlson, 1979; Corruccini and Handler, 1980; Corruccini et al., 1985; Brace et al., 1987, 1991; Larsen, 1997; González-José et al., 2005; Sardi et al., 2006; Pinhasi et al., 2008). Most of these studies focus on comparisons between hunter-gatherers and farmers, taking advantage of the general assumption that technological advances in the preparation of food that accompanied the origin of agriculture led to decreases in hardness and toughness in the diet of farmers versus foragers. This assumption is generally corroborated by evidence that hunter-gatherers typically have larger and more robust skulls (Brace et al., 1987, 1991; Larsen, 1995, 1997; Sardi et al., 2006). Larsen (1995), for instance, suggests that gracilization of the skull is a common and worldwide change characteristic of the transition from hunting-gathering to farming, and which was accompanied by earlier weaning ages (hence an increase in birth rates) and a general deterioration of dental health.

Another concern with comparative studies is the difficulty of separating the effects of shifts in populations (e.g., from migration) and demographic structure from changes in diet and food processing technology. The transition from foraging to farming was sometimes the result of group replacement, but in most cases the transition occurred via cultural diffusion or, by some combination of both processes (see Henry, 1989; Calafell and Bertranpetit, 1994; Larsen, 1995; Price et al., 1995; Chikhi et al., 1998; Gibbons, 2000; Richards et al., 2000; Semino et al., 2000; Eshed et al., 2004; Erickson et al., 2005; Bellwood, 2007; Pinhasi et al., 2008). It is therefore useful to examine morphological changes associated with technological transitions in populations for which there is independent evidence of genetic continuity. Although such samples are rare and often poorly studied, one interesting test case is the upper and middle Ohio valley, where years of meticulous archaeological research have yielded a rich skeletal record that spans the cultural sequence from the Indian Knoll-Archaic, to Woodland, to late prehistory periods. Importantly, this population was apparently stable (or at least at equilibrium in terms of genetic drift and migration) over the course of the tran-

TABLE 1. *Samples used in analysis*

Sample	Geographic origin	Economic strategy	Sex (m/f)	Total
Indian Knoll-Archaic	Upper-middle Ohio valley (USA)	Hunting-gathering	32/38	70
Woodland	Upper-middle Ohio valley (USA)	Horticulturalist	4/4	8
Late prehistoric	Upper-middle Ohio valley (USA)	Extensive farming	7/8	15
Pampa Grande	North western Argentina	Extensive farming	7/14	21
Bolivians	Southern Bolivia	Extensive farming	12/5	17
Total sex			62/69	131

sition from strict hunting-gathering, to horticulturalism, to extensive maize farming.

Cultural, archaeological, and historical records of native groups of the middle and upper Ohio valley region indicate that the transition from foraging to incipient horticulturalism occurred rapidly and in situ around 3,000 years before present (YBP) (Smith, 1989). Furthermore, the subsequent transition (1,200 YBP) from horticulturalism to the introduction of tropical domesticates, such as maize and beans, appears to have been produced by clustering of subpopulations into villages promoted by increased population sizes rather than by immigration of new people (Smith, 1989). According to Griffin (1983), the Indian Knoll-Archaic archaeological record can be seen as the culmination of ~3,000 years of relatively stable development shaped primarily by the internal dynamics of population growth and cultural differentiation.

Another advantage of studying morphological changes in the middle and upper Ohio valley population is that recent theoretical and methodological advances allow ancient populations to be evaluated in a manner directly comparable to contemporaneous populations (Williams-Blangero and Blangero, 1989; Relethford and Blangero, 1990). Tatarek and Sciulli (2000) addressed the issue of Ohio valley's population structure across different periods and detected a pattern of isolation-by-distance congruent with a balance between gene drift and migration. In agreement with previous archaeological studies (Griffin, 1983; Smith, 1989), the authors conclude that the differentiation of late prehistoric groups was accompanied by genetically similar populations occupying widely dispersed locations, rather than by interregional movement of people (Tatarek and Sciulli, 2000). Archaeologists suggest that the distribution of isotopic values associated with the appearance of maize farming indicate that an allopatric model might provide the better account of dietary change, i.e. some individuals changed their diets, experienced increased reproductive fitness, and replaced those who did not change their diets (Greenlee, 2001). Several careful studies have also tracked dietary changes in this population from archaeological evidence (e.g., Parmalee, 1975; Ford, 1979; Griffin, 1983; Muller, 1986; Yarnell, 1993), patterns of dental wear (Smith, 1984; Schmidt, 1998, 2001; Schmidt and Greene, 1998), and isotope analysis (Smith and Epstein, 1971; van der Merwe and Vogel, 1978; Bender et al., 1981; Buikstra et al., 1988; Greenlee, 2001). These studies suggest that changes in diets across these transitions were complex, probably involving shifts in hardness, toughness, and particle size.

In light of the general model outlined above regarding the effects of changes in diet on gracilization of the face, this study examines morphological changes in the craniofacial complex from the Ohio valley to test the extent to which dietary transformations in a single human population over time match the kinds of differences observed in experiments that compare animals fed hard

and soft foods. The goal is to shed light on the pattern and magnitude of epigenetic responses to changes in masticatory loading that occurred across the transition from hunting to farming and which include shifts in a wide variety of parameters such as hardness, toughness, particle size, and abrasiveness. In addition, geometric morphometric tools are used to measure and interpret the transformations observed in terms of independent and localized shape changes rather than in overall terms of "robusticity" versus "gracilization." The main hypothesis is that hunter-gatherers, horticulturalists, and farmers will differ primarily in those skull regions that are the location of masticatory muscle origins and insertions (e.g., zygomatic arch, temporal fossa, mandibular angle), or the site of high masticatory strains such as the temporo-mandibular joint, the alveolar crest, and the mandibular corpus.

## MATERIALS AND METHODS

### The sample

The study includes 131 adult individuals with no evidence of craniofacial deformation coming from the three main archaeological periods of the Ohio central valley, along with two north-Andean farmer groups used for statistical comparison as outgroups. The Ohio sample is stored at the Williams S. Webb Museum of Anthropology (Lexington, KY), whereas the two Andean groups belong to the Museum of La Plata (La Plata, Argentina). Previous studies concerning population genetics and dynamics can be found in Sciulli (1998, 2001), and Tatarek and Sciulli (2000) for the Ohio samples, and Sardi and Pucciarelli (2001), and Sardi (2002) for the Andean groups.

Sex and age were estimated following diagnostic traits outlined by Buikstra and Ubelaker (1994). Skulls were considered as "adults" if they had a completely obliterated spheno-occipital synchondrosis. Sample composition and further details are listed in Table 1. On each skull 46 cranial and 23 mandibular homologous three-dimensional landmark coordinates were recorded using a Microscribe G2X digitizer. The choice of landmarks listed in Table 2 is aimed to provide valuable, discrete biomechanical information: The transversal crosssection of the temporal fossa, which is informative of the relative space occupied by the temporal muscle, is represented by landmarks 33 (Zygomaxillare), 34 (MW1), 42 (MW2), and 43 (posterior point on the infratemporal fossa). The extension and orientation of the temporal muscle attach on the vault is represented by the relative position of the Stephanion (16) and the Enthomion (15). Both, the cross-section of the temporal fossa and the extension and orientation of the muscle attachment are informative about the relative position, orientation, shape, and size of the temporalis muscle, responsible for contraction of the jaw during mastication. Contraction of the temporal

TABLE 2. List of landmarks used in this study and assignment to first (*italic upper case*), second (*upper case*), and third (*lower case*) order landmark configurations of the skull and mandible

Landmark	No.	Definition	Region
Prosthion	1	The midline point at the most anterior point on the alveolar process of the maxillae	W/A/aa
Subspinale	2	The most posterior point in the concavity between the anterior nasal spine and prosthion, i.e., apical base	W/F, A/mf, aa
Nariale	3	The most inferior point on the nasal aperture	F/mf
Alare	4	The most lateral point on the margin of the nasal aperture	W/F/mf
Nasion	5	The midline point where the two nasal bones and the frontal intersect	W/F/sf
Asterion	6	The point where the lamboidal, parietomastoid, and occipitomastoid sutures meet	W/NC/ncv, ncb
Euryon	7	The points of greatest breadth of the brain case perpendicular to the median sagittal plane. The point is taken on the parietal eminences above the temporals	W/NC/ncv
Pterion	8	Is a region, rather than a point, where the frontal, temporal, parietal, and sphenoid meet on the side of the vault	W/NC/ncv
Zygion	9	The point of maximum lateral extent on the lateral surface of the zygomatic arch	M/mtf
Orbitale	10	The lowest point on the orbital margin	W/F/mf
Maxillofrontale	11	The point where the anterior lacrimal crest of the maxilla meets the frontomaxillary suture	W/F/sf
Orbitale superior	12	The highest point on the orbital margin	W/F/sf
Ectoconchion	13	The most lateral point on the orbital margin (independent of the sagittal plane)	F/sf
Frontomalare orb.	14	The point where the frontozygomatic suture crosses the inner orbital rim	F/sf
Enthomion	15	Parietal notch	NC/ncv
Stephanion	16	The point where the coronal suture crosses the temporal line	W/M/mtf
Glabella	17	The most anterior midline point on the frontal bone, usually above the frontonasal suture	W/NC/ncv
Metopion	18	Ectocranial midline point on the frontal where the frontal's elevation above the chord from nasion to bregma is greatest	NC/ncv
Bregma	19	The ectocranial point where the coronal and sagittal sutures intersect	W/NC/ncv
Vertex	20	The superior point of the cranium in the midsagittal contour when the skull is in Frankfurt Horizontal	NC/ncv
Lambda	21	Point of the intersection of the sagittal and lamboidal sutures in the medial sagittal plane	W/NC/ncv
Opisthocranion	22	The posterior-most point of the skull in the medial sagittal plane. It is the point at the farthest chord length from glabella	W/NC/ncv
Inion	23	An ectocranial midline point at the base of the external occipital protuberance. It is the point at which the superior nuchal lines merge in the external occipital protuberance	W/NC/ncv, ncb
Basion	24	The midline point on the anterior margin of the foramen magnum	NC/ncb
Hormion	25	The most posterior midline point on the vomer	W/NC/ncb
AM1	26	Point on the outer surface of the alveolar margin of the maxilla between C and P3	W/A/aa
IAM1	27	Point on the inner surface of the alveolar margin of the maxilla between C and P3	A/aa
AM2	28	Point on the outer surface of the alveolar margin of the maxilla between P4 and M1	W/A/aa, ap
IAM2	29	Point on the inner surface of the alveolar margin of the maxilla between P4 and M1	A/aa, ap
AM3	30	Point on the outer surface of the alveolar margin of the maxilla between M2 and M3	W/A/ap
IAM3	31	Point on the inner surface of the alveolar margin of the maxilla between M2 and M3	A/ap
Alveolar point	32	Posterior limit of the maxillary alveolar arch at the pterygo-alveolar suture	W/A/ap
Zygomaxillare	33	Lower border of the zygomatic synchondrosis	W/M/mtf
MW1	34	Most inferior point in the sphenotemporal crest in the great wing of the sphenoid bone	W/M/mtm
PMp	35	Most anterior point on the lamina of the greater wings of sphenoid	W/F/mf
Glenoid fossa	36	Posterior border of the glenoid cavity	W/M/mtm
Inferior glenoid fossa	37	Most inferior point on the glenoid fossa	M/mtm
Ant preglenoid plane	38	Most anterior point on the preglenoid plane	W/M/mtm

TABLE 2. (Continued)

Landmark	No.	Definition	Region
Lateral glenoid fossa	39	Most lateral point on the border of the glenoid cavity	W/M/mtm
Medial glenoid fossa	40	Most medial point on the border of the glenoid cavity	W/M/mtm
Jugale	41	The point in the depth of the notch between the temporal and frontal processes of the zygomatic	W/M/mtm
MW2	42	Most internal-inferior point on the zygomatic arch (ortogonal to sagittal plane) at the level of MW1	W/M/mtm
Post infratemp fossa	43	Most posterior point in the infratemporal fossa	W/M/mtm
Porion	44	The uppermost point on the margin of the external auditory meatus	W/NC/ncb
Stylomastoid foramen	45	Stylomastoid foramen	W/NC/ncb
Mastoidale	46	The most inferior point on the mastoid process	W/NC/ncb
Condylion medial	47	The most medial point on the mandibular condyle	MD/mdr
Condylion lateral	48	The most lateral point on the mandibular condyle	MD/mdr
Condylion posterior	49	The most posterior point on the mandibular condyle	MD/mdr
Condylion anterior	50	The most anterior point on the mandibular condyle	MD/mdr
Condylion superior	51	The most superior point on the mandibular condyle	MD/mdr
Deepest point AR	52	The deepest point on the curvature between the mandibular condyle and the coronoid process	MD/mdr
Coronoid	53	The most superior point on the coronoid process	MD/mdr
Most ant. point AR	54	The most anterior point on the posterior border of the ascending ramus	MD/mdr
Gonion	55	The point along the rounded posteroinferior corner of the mandible between the ramus and the body	MD/mdr
Border mental	56	The point on the external alveolar border at the level of the foramen mental.	MD/mdc
Mental foramen	57	The highest point on the border of the foramen mental	MD/mdc
Interior mental foramen	58	The point corresponding to the mental foramen in the internal face of the mandibular corpus.	MD/mdc
Gnathion	59	The most inferior midline point on the mandible	MD/mdc
Infradentale	60	The midline point at the most anterior point on the alveolar process of the mandible	MD/mdc
Inner infradentale	61	The midline inner point at the most anterior point on the alveolar process of the mandible	MD/mdc
Torus mandibular	62	The sagittal point on the torus mandibular	MD/mdc
AM1md	63	Point on the outer surface of the alveolar margins of the mandible between C and P3	MD/mdc
IAM1md	64	Point on the inner surface of the alveolar margins of the mandible between C and P3	MD/mdc
AM2md	65	Point on the outer surface of the alveolar margins of the mandible between P4 and M1	MD/mdc
IAM2md	66	Point on the inner surface of the alveolar margins of the mandible between P4 and M1	MD/mdc
AM3md	67	Point on the outer surface of the alveolar margins of the mandible between M2 and M3	MD/mdc
IAM3md	68	Point on the inner surface of the alveolar margins of the mandible between M2 and M3	MD/mdc
Mylohyoid foramen	69	The highest point on the border of the milohioid foramen	MD/mdc

Bilateral landmarks were digitized on the left side. W = whole skull, NC = neurocranium, ncb = base, ncv = vault, F = face, sf = superior face, mf = middle face, A = alveolar, aa = anterior alveolar, ap = posterior alveolar, M = masticatory, mtf = masticatory, temporal fossa, mtm = masticatory, temporo-mandibular joint, MD = mandible, mdr = mandibular ramus, mdc = mandibular corpus.

muscle elevates the mandible, and the somewhat horizontal fibers of the posterior part of the muscle retract the mandible. In humans, the temporalis muscle is the most powerful one of the body. The temporo-mandibular joint, a structure that reflects the area occupied by the joint itself but also refers to the insertion region of the temporomandibular ligament was studied after landmarks 36, 37, 39, and 40, and the extension of sphenomandibular ligament, which runs from the spine of the sphenoid bone to the lingula of the mandible was represented by the landmark 38 (anterior point on the preglenoid plane). These ligaments restrict movements of the mandible, and help maintain joint stability on the balancing side when high bite forces can cause TMJ distraction.

Alveolar morphology is represented by Prosthion (1), Subspinale (2), AM1 (26), IAM1 (27), AM2 (28), IAM2

(29), AM3 (30), IAM3 (31), and Alveolar point (32) in the maxillary alveolar arch and by Infradentale (60), Inner infradentale (61), AM1md (63), IAM1md (64), AM2md (65), IAM2md (66), AM3md (67), and IAM3md (68), in the mandibular alveolar arch. Collectively, these landmarks are informative about the extension of the alveolar crest, and they are informative of putative decreasing of growth in this region due to low magnitudes and frequency of loadings.

Whereas analyzed independently or in relation to other cranial structures, these landmarks provide relevant information about force production and/or stress absorption.

The landmarks, summarized in Table 2, were divided into different subsets, which help to delimitate hierarchically nested ("first order," "second order," and

“third-order”) regions and structures of biomechanical interest. Also, this subdivision allows a more accurate, hierarchical representation of the morphological integration patterns of the human skull (Bastir and Rosas, 2005) than a more classical separation of structures at equally hierarchical levels. The nesting scheme is conceived to help in the detection of particular, localized changes that can be of utility to interpret the biomechanical behavior of the skull during mastication. The logic to divide the skull into parts reflecting functional demands is based on the pioneering work by Moss (1968). In Moss’ functional matrix hypothesis, the growth of the skeletal units is determined by the function of the soft tissues and functional spaces in which they are embedded. The functional matrix includes all the elements (organs, tissues, nerves, functional spaces, etc.) necessary to perform a function. The skeletal unit supports and protects its specific functional matrix (Moss, 1979). According to this hypothesis, it is expected that skeletal elements that are part of the same functional matrix will be more highly integrated than they will be with traits of a different functional matrix.

The skull is hierarchically organized (Bastir and Rosas, 2005) into two major functional units or components: the facial component composed of the bones surrounding the nasal, oral, and pharyngeal capsules, and a neurocranial component composed of the cranial vault and basicranium that surrounds the brain (Willmore et al., 2006). These two functional components can be further subdivided following different criteria (e.g. see Cheverud, 1982, 1995; Pucciarelli et al., 1990; Willmore et al., 2006). Here we have adopted a hierarchically nested approach by subdividing a first-order landmark subset (the whole skull) into five second-order subsets: neurocranial, face, alveolar, masticatory, and mandibular (see Fig. 1). Furthermore, these second-order subsets are subdivided into third-order ones: the neurocranial into cranial vault and basicranium; the facial into superior and middle face; the alveolar into anterior and posterior alveolar; the masticatory into temporal-fossa and temporo-mandibular joint; and the mandibular into mandibular corpus and mandibular ramus. Unfortunately, mandibles were not available for the Andean series, so comparisons concerning the lower face were limited to the Ohio valley sample.

### The Ohio valley sample

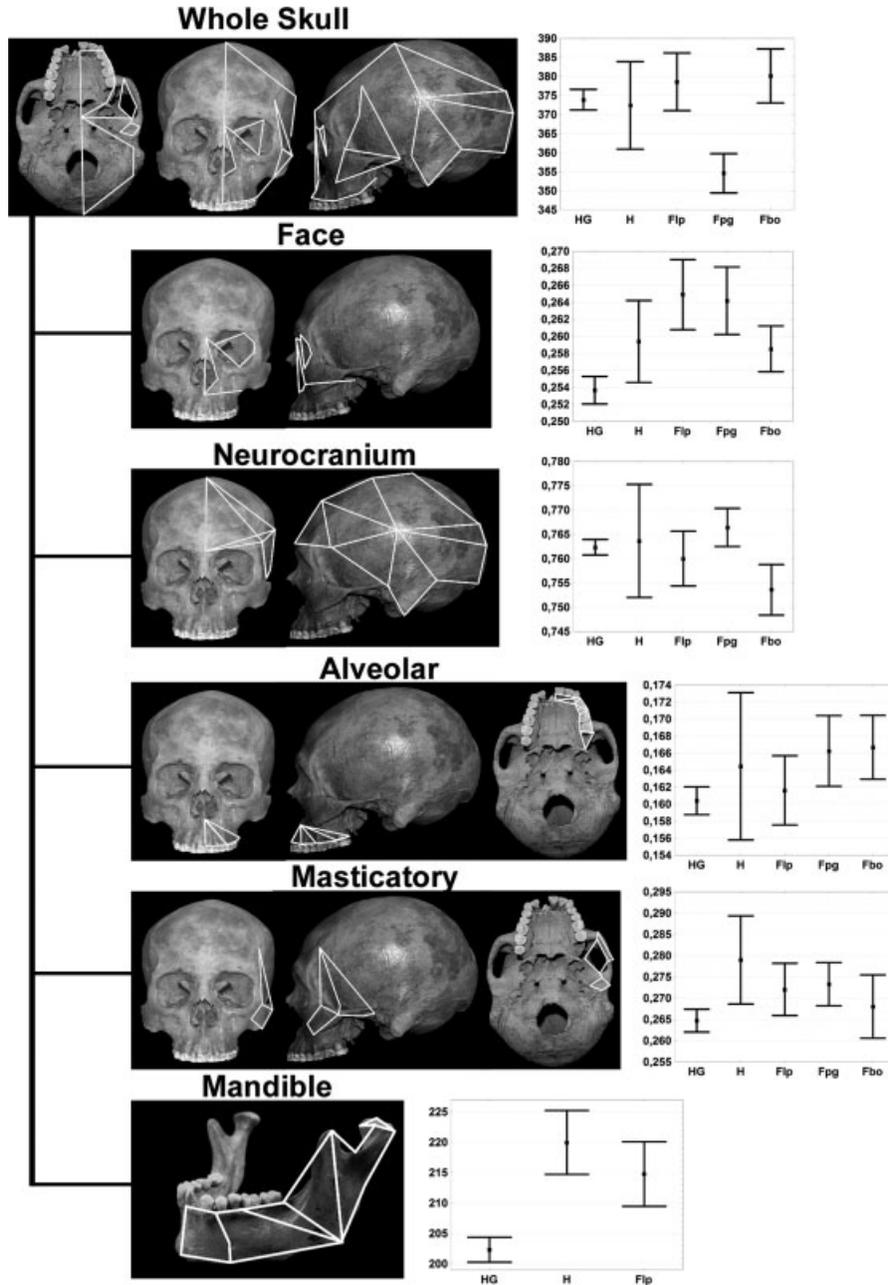
The Indian Knoll-Archaic (hunter-gatherers, HG) period begins 10,000 YBP and ends with the invention of pottery ~3,000 YBP (Winters, 1969; Jennings, 1974; Cassidy, 1984; Hill, 2003). Over time, Indian Knoll-Archaic hunter gatherers transitioned from nomadic to semi-sedentary lifestyles, with subsequent increases in population size and ceremonialism (Winters, 1969; Janzen, 1977; Muller, 1986; Hill, 2003). They hunted fauna such as deer, collected mussels and foraged from a wide variety of plants including seeds, berries, nuts, squash, and gourds (Griffin, 1983). They also cultivated local starchy oily seeds such as sumpweed and sunflowers (Yarnell, 1993).

The Woodland (horticulturalists, H) period is divided into the early/middle Woodland and late Woodland phases. The first period, from 3,000 to 1,500 YBP (Griffin, 1983; Hill, 2003), is characterized by an increase in cultural complexity, which is evident in

trade, burial styles, settlement patterns, and social organization (Brose, 1979; Muller, 1986; Clay, 1990; Hicks, 1992; Hill, 2003). Pottery and domestication of cultigens as squash and starchy foods intensified during this time (Griffin, 1983; Muller, 1986). During this period seed and nut gathering accompanied gardening (Ford, 1979), and there is also evidence for the consumption of deer and small mammals (Parmalee, 1975), as well as turtle, fish, and mussels. These foods were probably prepared with stone tools like those of the late Archaic, but they may also have been further processed via boiling in ceramic vessels, which first appear in this region during this period (Kellar, 1983). Thus, food processing appears to have become more intensive from the late Archaic to Woodland. The early/middle Woodland phase is represented by two cultural complexes: Adena and Hopewell. The late Woodland occupations emerged between 1,500 to 1,200 YBP (Griffin, 1983; Hill, 2003). This period was characterized by a mixed horticulturalist economy. Even though the earliest maize consumption is documented for this period, late Woodland groups are not considered to be exclusive and extensive agriculturalists (Muller, 1986; Bush, 1994; Schmidt, 1998). It is assumed that by the end of this period, maize had largely replaced the native crops and wild plants resources in Ohio valley’s archaeobotanical assemblages (Greenlee, 2001).

Finally, the late prehistory (farmers,  $F_{LP}$ ) period is represented by the Mississippian transition, which spanned from 1,200 to 500 YBP (Griffin, 1978; Hill, 2003). These groups were sedentary and occupied densely populated areas (Griffin, 1983; Muller, 1986; Hill, 2003). Maize was the most important subsistence resource (Cassidy, 1984; Larsen, 2002), and concomitant with its domestication (Greenlee, 2001) there is a decrease in the frequency of cultivated native and wild plant resources like beans, squash, sunflower, and chenopod. Conversely, the transition in other regions was characterized by coexistence among maize and indigenous plants, which apparently continued to contribute significant amounts to subsistence even after maize became a stable crop (Greenlee, 2001). Also, they consumed animals such as deers, birds, fishes, reptiles, mussels, and amphibians (Parmalee, 1975). Beginning with the Woodland phases, the proliferation, diversification, and increasing complexity of pottery highlights the importance of cooking and processing foods that were acquired in the Ohio valley.

In addition to archaeological evidence as an indirect indicator of an increase in food processing, dental microwear data also indicate a trend to consume more processed food. According to previous studies in these groups, there is a trend toward softer/less abrasive and more cariogenic diet in the late Archaic–Woodland–late prehistory transition (Smith, 1984; Sciulli, 1997; Schmidt, 1998, 2001; Schmidt and Greene, 1998). The first diet probably consisted of wild plant and riverine resources contaminated by sand, which is a good candidate for causing the wide scratches and rapid wear observed on the Archaic teeth. The Woodland diet was probably more processed, so grit was more effectively removed and the scratches are less common. Alternatively, food processing tends to soften foods, so a shift in consumed food resources (nuts and starchy/oil seeds) rather than food processing likely accounts for the change seen in the pattern of microwear, specifically in the increasing of pits (Sciulli, 1997, Schmidt, 1998, 2001). Finally, the late prehistorical diet was almost based on maize agriculture (very cariogenic) since neither scratches nor pits are common, but the fre-



**Fig. 1.** Wireframes corresponding to the first and second-order configurations along with variation in size for each subset and each sample. Mean (mean of the centroid size) and whiskers (95% confidence interval) are shown in the right side of the figure. For the second order structures, size is represented relative to total centroid size. HG: hunter-gatherers/foragers from the Indian Knoll-Archaic period, H: horticulturalists from the Woodland period, F: maize farmers from the Ohio's late prehistoric ( $F_{LP}$ ), Pampa Grande ( $F_{PG}$ ), and Bolivia ( $F_{BO}$ ).

quency of caries is high (Schmidt, 1998, Schmidt and Greene, 1998). The absence of pits and scratches strongly suggest a trend towards more food processing and the subsequent softening of diet.

Even though there are no definitive measures concerning the hardness, stiffness, and toughness suffered by people from the Ohio valley at any period, we assume that all the archaeological and dental evidence above points to a biomechanical context where foods eaten by farmers required less occlusal force and fewer chewing cycles per day.

Samples from Pampa Grande and Bolivia correspond to Andean pastoralists and agriculturalists sedentary groups. The Bolivian ( $F_{BO}$ ) sample is formed primarily by peasant pastoralists deriving their livelihood from herds of alpaca and llama, and also by subsistence agriculturalists, the former in the altiplano and the latter in the sierras (between 2,000 and 3,000 m. of altitude). Generally, potatoes were the stable crop, although quinoa was the most important grain. Pastoralism was important not for food, but for transport and for the use of wool to make clothes. The Pampa Grande ( $F_{PG}$ ) series

was assigned to the "La Candelaria" culture, which reached his maximum development among 200 and 1000 AD on the Andean part of northwestern Argentina. La Candelaria is represented by many archaeological sites like Sierras de Medina, Trancas, El Cadillal, Vipos, San Pedro de Colalao, La Sala, Raco, Sierras de San Javier, Horco Molle y Yerba Buena (González and Pérez, 1987). Archaeological research on these sites indicates a sedentary life style and an economy based on llama pastoralism and maize as the main domesticated crop.

### Statistical analysis

Landmark configurations were superimposed by generalized Procrustes analysis (GPA, Rohlf and Slice, 1990; Goodall, 1991) using the IMP-Simple3D software (Sheets, 2001; IMP-Simple3D). Data used in this study were collected by one of us (R.G.J.) and the effect of intraobserver error was evaluated by collecting 10 observations of a single specimen. After the Procrustes superimposition, the Euclidean distance of each landmark to its respective centroid was computed and landmark deviations were calculated relative to the landmark mean. Following Singleton (2002), mean deviations were calculated for individual landmarks and subsequently averaged to give a mean deviation across all landmarks. The Singleton (2002) approach is based on a One-way Analysis of Variance (ANOVA) performed for each landmark. The root of the within-groups mean squares (root mean square error) is an estimation of the intraobserver error (Sokal and Rohlf, 1995). Mean deviation for the observer of the present analysis (RGJ) was 0.370 mm. Considering the relatively large size of the structures studied here, these margins of error were considered acceptable.

Superimposition was made independently for each of the landmark configurations depicting first, second, and third order subsets of landmarks described in Table 2. GPA removes the effects of translation, rotation, and scaling (Rohlf and Slice, 1990). After superimposition, pure shape information is preserved in the specimens' aligned landmarks, and size is calculated as the centroid size, the square root of the summed distances between each landmark coordinate, and the centroid (Dryden and Mardia, 1998). The Procrustes superimposition therefore removes any effects of scale but not the allometric shape variation that is related to size. To remove correlations among shape variables due to allometry, we computed the residuals of the regional Procrustes coordinates on centroid size and standardized each dataset by its mean centroid size using IMP-ThreeDStand6 (Sheets, 2004; IMP ThreeDStand6). The fitted coordinate configurations resulting from these procedures were then placed in the denominated Kendall's shape space (Rohlf, 1996). As this shape space is non-Euclidean, further statistical analyses were performed by projecting the coordinates into a linear tangent space (Dryden and Mardia, 1998).

Interpopulation differences on size were studied after ANOVA tests computed on the centroid sizes corresponding to each landmark subset. Superimposed landmark coordinates were used as the input data for three independent analyses to quantify differences among subsamples. First, the Goodall's *F*-test was computed to test for overall shape differences between groups (Goodall, 1991). This statistic is based on the ratio of the squared Procrustes distance between the means of each sample, to the sum of the squared Procrustes distance of each specimen to its group mean. Assessments of mean shape

differences were made between pairwise samples for the whole skull, as well as for all the landmark subsets using the Goodall's *F*-test bootstrap version included in the Simple 3D (Sheets, 2001; IMP-Simple3D). This test gives an actual metric of shape difference, rather than a visual description.

Second, a principal component analysis for the whole skull and for each cranial region was performed to visualize shape differences using the program 3DPCA (Sheets, 2001; IMP-ThreeDPCA6), and displayed as scatterplots of the first two principal components. Wireframe deformations corresponding to extreme negative and positive values of the first two principal components corresponding to the whole skull configuration were used to detect visually the magnitude and direction of shape change. Both Goodall's *F* test and principal component analysis were repeated using the superimposed and free-of-allometry landmark coordinates. Even though allometry effects should be addressed departing from a widest sampling including variation at the ontogenetic, evolutionary (e.g. among populations, regions, etc.), and static (e.g. within-population) we present here some specific analyses comparing pure-shape versus allometry-free results to preliminary detect putative trends regarding the allometric component of response to masticatory loadings.

Finally, Euclidean Distance Matrix Analysis (EDMA) was computed using WinEdma (Cole, 2002). Unlike GPA superimposition methods, EDMA is a coordinate-free approach which quantifies differences in particular interlandmark distances and tests their statistical significance (Lele and Richtsmeier, 1995, 2001; Richtsmeier et al., 2002). Estimation of differences in shape using the WinEDMA program (Cole, 2002) is accomplished by first computing a form matrix of all pairwise interlandmark ratios. A shape matrix is then calculated by dividing the form matrix by a scaling factor, in this case the geometric mean, computed as the *n*th root of the product of all interlandmark distances. The shape matrices were then compared by the calculation of shape difference matrices by subtracting one shape matrix from the other. A difference not significant from 0 indicates equal shapes. In addition, EDMA indicates which interlandmark distances are contributing most to the shape change. Thus, the scaled interlandmark differences found among populations were then used to explore localized skull shape changes (Lele and Richtsmeier, 1995, 2001; Richtsmeier et al., 2002). The statistical significance of localized shape differences was tested using a nonparametric bootstrap procedure to calculate the 100 (1 -  $\alpha$ )% confidence interval for each size-corrected linear distance (Lele and Cole, 1996; Lele and Richtsmeier, 2001). Confidence intervals were obtained after 999 iterations and  $\alpha = 0.05$ . A particular interlandmark distance is considered to be equal in two given samples if the resulting interval contains the value zero. Otherwise, the null hypothesis of similarity is rejected and it is assumed that a significant shape difference exists at the  $\alpha$  level in that specific region (Lele and Cole, 1996). For example, if no difference exists between two landmarks, the relevant shape difference matrix value would be 0. Consequently, values greater or lower than 0 indicate that distances between landmarks differ. Thus, in a shape difference matrix between Groups A and B, a value of 0.05 for any interlandmark distance indicates that the distance is 5% longer in A. For simplicity, these comparisons were limited to the Ohio populations and intended to detect the localization and magnitude of change among them.

TABLE 3. Mean centroid sizes for each cranial region and sample studied, and ANOVA test to evaluate intergroup size differences

Region	Mean CS (HG)	Mean CS (H)	Mean CS ( $F_{LP}$ )	Mean CS ( $F_{PG}$ )	Mean CS ( $F_{BO}$ )	<i>F</i>	<i>P</i>
Whole skull (1st order)	373.89	372.42	378.58	354.59	380.14	<b>14.50</b>	<b>0.00</b>
Face (2d order)	94.84	96.56	100.28	93.67	98.27	<b>9.97</b>	<b>0.00</b>
Sup. Face (3rd order)	49.43	50.32	51.33	49.53	50.04	2.41	0.05
Middle Face (3rd order)	51.98	52.61	53.56	49.17	51.89	<b>6.03</b>	<b>0.00</b>
Neurocranium (2d order)	285.02	284.31	287.69	271.76	286.43	<b>10.64</b>	<b>0.00</b>
Vault (3rd order)	247.65	246.62	248.06	236.27	249.41	<b>9.45</b>	<b>0.00</b>
Base (3rd order)	114.53	111.77	117.16	109.44	117.35	<b>9.28</b>	<b>0.00</b>
Alveolar (2d order)	59.95	61.19	61.19	58.96	63.33	<b>5.63</b>	<b>0.00</b>
Ant. Alveolar (3rd order)	34.67	35.91	35.77	35.09	36.78	2.29	0.06
Post. Alveolar (3rd order)	28.36	27.95	29.53	25.67	29.16	<b>7.23</b>	<b>0.00</b>
Masticatory (2d order)	98.99	103.98	102.98	96.95	101.93	<b>4.25</b>	<b>0.00</b>
Temporal Fossa (3rd order)	81.48	86.29	86.14	82.70	87.63	<b>5.03</b>	<b>0.00</b>
Temp-mand-joint (3rd order)	22.15	21.28	21.36	21.14	21.48	<b>4.92</b>	<b>0.00</b>
Mandible (2d order)	205.01	211.81	216.44	n/a	n/a	<b>6.94</b>	<b>0.00</b>
Corp. Mandible (3rd order)	70.01	73.19	73.05	n/a	n/a	<b>5.02</b>	<b>0.01</b>
Ram. Mandible (3rd order)	68.62	71.67	73.03	n/a	n/a	<b>5.60</b>	<b>0.01</b>

Significant differences are bolded. HG: hunter-gatherers/foragers from the Indian Knoll-Archaic period, H: horticulturalists from the Woodland period, F: maize farmers from the Ohio's Late Prehistoric ( $F_{LP}$ ), Pampa Grande ( $F_{PG}$ ), and Bolivia ( $F_{BO}$ ).

RESULTS

Figure 1 depicts the variations in size for the first and second-order configurations for each sample. The only population that differs significantly in terms of overall size is the Farmers from Pampa Grande. An inspection of size variation across the second order configurations reflects that the face, masticatory, and mandibular regions are significantly smaller in the hunter-gatherer group. In addition, statistical differences in size computed after an ANOVA are presented in the Table 3. Size differences are significant for all areas of the skull, excepting the superior face and the anterior alveolar regions. In agreement with Figure 1, Table 3 confirms the fact that hunter-gatherers have generally smaller craniofacial structures, except for the third-order configuration depicting the temporo-mandibular joint, which is relatively larger in the hunter-gatherers.

Table 4, which summarizes shape differences among groups using Goodall's bootstrap *F*-tests (before and after removing the allometric component of shape variation), indicates that the samples differ in overall shape. In general terms, and as expected, between-group differences are greater for the whole-skull landmark configuration, remain strong for most of the comparisons across the first-order configurations, and tend to decrease in the second and third-order comparisons. The most important exception concerns the horticulturalist and farmer samples from the Ohio valley (Woodland and late prehistoric respectively), which hardly differ except in the temporo-mandibular joint region. An important general result observed in Table 4 is that, among the lower order landmark subsets, differentiation is greater in the masticatory and its two derivatives: the temporal fossa and the temporo-mandibular joint.

A more detailed inspection of pairwise comparisons indicate that shape differences are significant between hunter-gatherers (HG) and the two south Amerindian farmers samples ( $F_{PG}$ ,  $F_{BO}$ ) for all the first-order regions considered. Differences among the Ohio's horticulturalists and farmers and the two South Amerindian outgroups are also significant for most structures but do not achieve the degree of differentiation evident in the HG- $F_{PG}$  and HG- $F_{BO}$  comparisons in terms of number and order of regions and in the magnitude of the *F*-ratios. The second order area that most differs among groups is

the masticatory complex, with the greatest differences among the hunter-gatherers and the farmers. Within the masticatory apparatus, the shape of the temporal fossa and temporal muscle origin explain the largest amount of between-group variation. In addition, the Goodall's *F*-test indicates that the largest *F*-ratio is found for the masticatory and the temporal-fossa second and third-order regions, respectively, in comparisons involving the hunter-gatherers versus the South Amerindian farmers. Goodall's *F*-test performed on the mandibles reflects that they are not different among the Ohio samples. However, analysis of the third-order regions reflect some significant differences on the mandibular corpus and the mandibular ramus for the hunter-gatherer-farmer comparison.

Results concerning the Goodall's *F* test computed on the allometry-free coordinates are much the same as the previous results. This is expected due to limited variation in size of the whole sample. The alveolar component seems to be the most affected by allometric effects: it shows significant differences in seven out of ten pairwise comparisons, but only four remain significant after controlling for allometry.

Figure 2 presents the projection of the samples along the first two principal components for the whole skull as well as for the first and second-order (Fig. 2a) and the third-order (Fig. 2b) configurations. These scatterplots suggest that the greatest differentiation among groups is found for the whole skull as a whole, primarily because of differences in the neurocranium rather than within the face. Whatever the level observed, the greatest differentiation tends to be in the masticatory structures. Further, within this region, between-group differences are primarily attributable to variation in the third-order temporal-fossa subset (Fig. 2b). However, these plots also show that there is a great amount of overlap of shape configurations among the groups for the different regions of the skull. Thus, when considered in conjunction with the results of the Goodall's *F* tests, the principal component analysis indicates that, even though some comparisons suggest between-group differences in the vault, cranial base, or face, the greatest degree of differentiation among hunter-gatherers in one hand, and horticulturalists and farmers in the other hand is concentrated in the temporal fossa. Beyond the differentiation level observed at the temporal fossa, the neurocranium as a

TABLE 4. Goodall's *F*-test results for shape differences between groups for all regions of the skull and mandible

Region	HG— <i>H</i>	HG— <i>F</i> <sub>LP</sub>	HG— <i>F</i> <sub>PG</sub>	HG— <i>F</i> <sub>BO</sub>	<i>H</i> — <i>F</i> <sub>LP</sub>	<i>H</i> — <i>F</i> <sub>PG</sub>	<i>H</i> — <i>F</i> <sub>BO</sub>	<i>F</i> <sub>LP</sub> — <i>F</i> <sub>PG</sub>	<i>F</i> <sub>LP</sub> — <i>F</i> <sub>BO</sub>	<i>F</i> <sub>PG</sub> — <i>F</i> <sub>BO</sub>
Whole skull (1st order)	<b>4.86**</b>	<b>7.55**</b>	<b>11.50**</b>	<b>9.22**</b>	1.1	<b>2.52**</b>	<b>4.90**</b>	<b>3.12**</b>	<b>4.89**</b>	<b>4.9**</b>
	<b>4.73**</b>	<b>6.48*</b>	<b>11.88**</b>	<b>9.16**</b>	1.2	<b>2.49**</b>	<b>2.49**</b>	<b>3.06**</b>	<b>4.31**</b>	<b>4.98**</b>
Face (2d order)	1.47	<b>4.60**</b>	<b>4.83**</b>	<b>8.91**</b>	1.15	<b>2.86**</b>	<b>4.94**</b>	<b>2.57**</b>	<b>2.87**</b>	<b>6.01**</b>
	1.41	<b>2.45*</b>	<b>4.28**</b>	<b>8.30**</b>	0.98	<b>3.14**</b>	<b>5.16**</b>	<b>2.30*</b>	<b>2.25*</b>	<b>6.23**</b>
Sup. Face (3rd order)	0.63	<b>2.64**</b>	<b>3.06**</b>	<b>8.67**</b>	0.61	0.42	<b>3.39**</b>	1.02	<b>4.34**</b>	<b>6.55**</b>
	0.65	<b>3.02*</b>	<b>3.01*</b>	<b>7.96**</b>	0.65	0.44	<b>3.47*</b>	1.17	<b>4.54**</b>	<b>6.54**</b>
Middle Face (3rd order)	1.67	<b>3.27**</b>	<b>3.17**</b>	<b>4.49**</b>	0.95	<b>4.65**</b>	<b>2.91**</b>	<b>2.95**</b>	1.15	<b>2.34*</b>
	2.29	<b>2.52*</b>	<b>2.76*</b>	<b>3.34*</b>	0.7	<b>5.04**</b>	<b>3.15*</b>	<b>3.13*</b>	1.6	<b>2.36*</b>
Neurocranium (2d order)	<b>4.70**</b>	<b>6.95**</b>	<b>8.85**</b>	<b>4.59**</b>	1.26	<b>2.35**</b>	<b>5.18**</b>	<b>1.74**</b>	<b>3.89**</b>	<b>5.16**</b>
	<b>4.58**</b>	<b>5.71**</b>	<b>9.18**</b>	<b>4.56**</b>	1.34	<b>8.17**</b>	<b>4.27**</b>	1.37	<b>3.48**</b>	<b>5.26**</b>
Vault (3rd order)	<b>4.45**</b>	<b>7.52**</b>	<b>9.15**</b>	<b>5.22**</b>	1.22	<b>2.05**</b>	<b>4.84**</b>	1.32	<b>3.41**</b>	<b>4.98**</b>
	<b>4.33**</b>	<b>7.77**</b>	<b>4.71**</b>	<b>5.45**</b>	1.29	1.94	<b>3.91**</b>	1.37	<b>3.38**</b>	<b>2.86**</b>
Base (3rd order)	<b>2.40**</b>	<b>2.00*</b>	<b>6.85**</b>	<b>2.87**</b>	0.93	<b>2.55**</b>	<b>3.87**</b>	1.48	<b>2.73**</b>	<b>5.41**</b>
	<b>2.27**</b>	1.16	<b>7.28**</b>	<b>2.91**</b>	1.06	<b>2.62*</b>	<b>3.72**</b>	1.59	1.84	<b>5.69**</b>
Alveolar (2d order)	<b>1.73*</b>	<b>2.01**</b>	<b>4.82**</b>	<b>2.07**</b>	0.94	<b>1.77*</b>	1.33	<b>4.44**</b>	<b>2.28**</b>	1.43
	1.45	1.79	<b>4.34**</b>	<b>2.29*</b>	0.96	1.85	1.36	<b>4.72**</b>	<b>3.19**</b>	1.57
Ant. Alveolar (3rd order)	<b>2.47**</b>	<b>2.46**</b>	<b>3.10**</b>	1.73	1.19	<b>2.33**</b>	<b>2.16*</b>	<b>4.63**</b>	<b>3.05**</b>	1.68
	1.92	2.25	<b>3.23*</b>	1.81	1.25	2.19	1.69	<b>4.96**</b>	<b>2.69*</b>	1.85
Post. Alveolar (3rd order)	<b>3.02**</b>	1.81	1.66	1.79	0.98	<b>2.55*</b>	1.57	<b>2.27*</b>	1.46	0.32
	<b>2.76*</b>	1.58	1.27	1.16	1.04	2.5	1.58	<b>2.40*</b>	1.55	0.29
Masticatory (2d order)	<b>3.82**</b>	<b>4.04**</b>	<b>27.65**</b>	<b>25.71**</b>	0.97	<b>7.20**</b>	<b>6.94**</b>	<b>11.44**</b>	<b>10.14**</b>	<b>2.46**</b>
	<b>3.78**</b>	<b>3.82**</b>	<b>27.67**</b>	<b>29.62**</b>	0.99	<b>23.95**</b>	<b>6.64**</b>	<b>10.27**</b>	<b>9.58**</b>	<b>2.35*</b>
Temporal Fossa (3rd order)	<b>4.11**</b>	<b>2.80**</b>	<b>31.81**</b>	<b>27.26**</b>	1.01	<b>7.91**</b>	<b>7.60**</b>	<b>15.45**</b>	<b>12.84**</b>	<b>3.26**</b>
	<b>4.65**</b>	<b>3.57**</b>	<b>33.62**</b>	<b>29.81**</b>	1.03	<b>6.78**</b>	<b>6.76**</b>	<b>13.71**</b>	<b>11.44**</b>	<b>3.51**</b>
Temp-mand-joint (3rd order)	<b>4.28**</b>	<b>8.09**</b>	<b>3.60**</b>	<b>4.27**</b>	<b>2.52*</b>	<b>2.35*</b>	<b>2.56*</b>	<b>3.48**</b>	1.93	0.65
	<b>3.50**</b>	<b>6.52**</b>	<b>3.50*</b>	<b>3.47*</b>	<b>2.75*</b>	2.02	<b>2.64*</b>	<b>3.01*</b>	2.03	0.42
Mandible (2d order)	<b>1.71**</b>	<b>1.51**</b>	n/a	n/a	1.23	n/a	n/a	n/a	n/a	n/a
	1.59	1.72	n/a	n/a	1.32	n/a	n/a	n/a	n/a	n/a
Corp. Mandible (3rd order)	<b>1.54*</b>	0.64	n/a	n/a	0.86	n/a	n/a	n/a	n/a	n/a
	0.8	<b>2.06*</b>	n/a	n/a	1.17	n/a	n/a	n/a	n/a	n/a
Ram. Mandible (3rd order)	1.36	<b>3.40**</b>	n/a	n/a	<b>1.46*</b>	n/a	n/a	n/a	n/a	n/a
	1.01	<b>2.23*</b>	n/a	n/a	1.00	n/a	n/a	n/a	n/a	n/a

Bolded values are significant at  $P < 0.05$  (\*) or  $P < 0.01$  (\*\*). The first line indicates results from the superimposed configurations, whereas the second line corresponds to results after the removal of the allometric component of shape variation. HG: hunter-gatherers/foragers from the Indian Knoll-Archaic period, *H*: horticulturalists from the Woodland period, *F*: maize farmers from the Ohio's Late Prehistoric (*F*<sub>LP</sub>), Pampa Grande (*F*<sub>PG</sub>), and Bolivia (*F*<sub>BO</sub>).

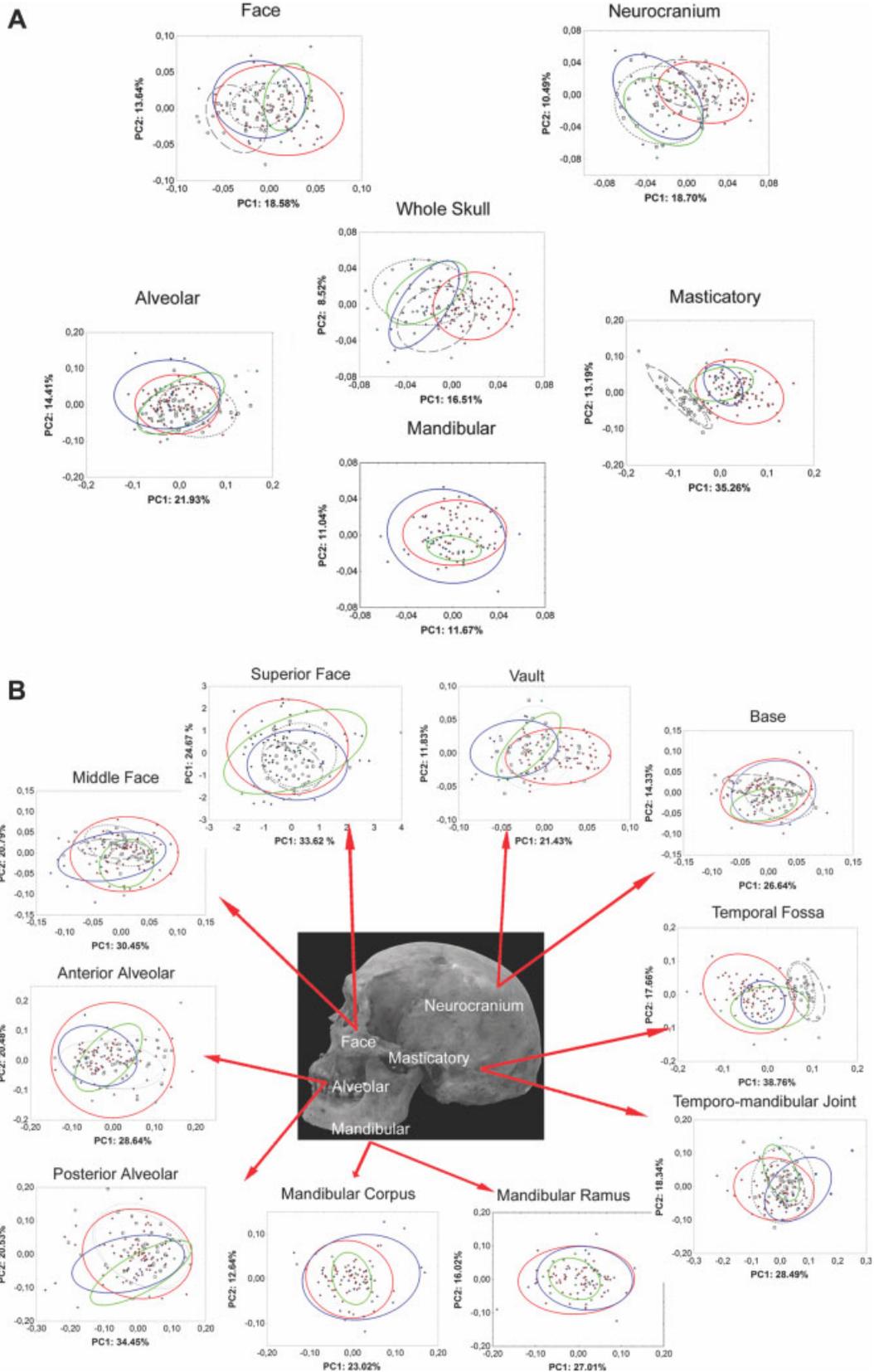
whole is also important in terms of discrimination among groups.

Figure 3 outlines the lateral, basicranial, and frontal wireframe views of the deformation polygons along the first two principal components for all the groups and for the whole-skull configuration. In addition, Figure 3 depicts main differences along the first principal component of the nonallometric dataset. Note that hunter-gatherers are represented on the maximum values of the first PC, whereas horticulturalists and farmers occupy the minimum values. In the lateral view, the hunter-gatherers present a forward and downward displacement of the stephanion landmark, which demarcates the anterosuperior origin of the anterior portion of the temporalis muscle. Also, positive values along the first PC show a greater distance among the anterior portion of the temporal fossa and the posterior border of the glenoid cavity. Further changes are observed in the frontal and basal views, where it is evident that both the temporal fossa and the glenoid cavity are placed more medially in the hunter-gatherers from the Indian Knoll series. In addition, the line delimited by the anterosuperior origin of the temporalis muscle (stephanion) and the zygomatic arch is relatively higher in the hunter-gatherers. Along the first PC, the groups are also differentiated in terms of the orientation of the lateral margin of the zygomatic in frontal view, which is oriented more vertically on the hunter-gatherers. Also, hunter-gatherers represented in the positive values of the first PC have a wider and

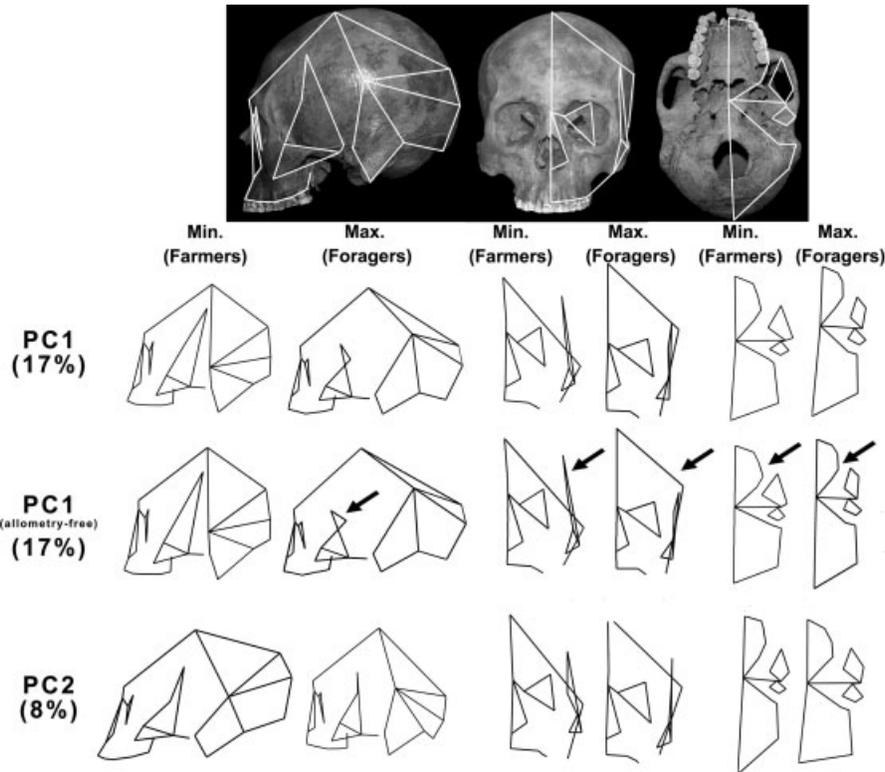
longer palate compared to horticulturalists and farmers. Removal of the allometric component results in some variations (marked by arrows in Fig. 3) on the alveolar component, which presents slightest differences in the lateral projection of the alveolar border among farmers and hunter-gatherers. In addition, removal of allometry derives in greater downward displacement of the stephanion. Note that the visualization of changes along both, the pure-shape and nonallometric first principal components, is coincident with results of the Goodall's *F* test presented in Table 4.

Further differences in facial projection along the second PC can be observed, such as variations in prognathism and height and length of the neurocranium. However, changes across the second PC do not differ significantly between hunter-gatherers and farmers.

Results corresponding to the EDMA analysis of shape differences between the three Ohio valley's groups are presented in Table 5 and illustrated in Figure 4. For simplicity, only the 10 greatest and significant interlandmark distances at the  $\alpha = 0.05$  level are shown for each pairwise comparison. For the HG-*H* and HG-*F*<sub>LP</sub> pairwise comparisons, the 10 greatest interlandmark relative distances are greater in HG than in *H* or *F*<sub>LP</sub>. However, the *H* versus *F*<sub>LP</sub> comparison shows relative interlandmark distances in both directions, greater distances in *H* or else *F*<sub>LP</sub>. The pattern of relative interlandmark dimensions is significantly different between the hunter-gatherers from Indian Knoll and the horticulturalists from



**Fig. 2.** Principal component scatterplot for the whole skull and for all the regions of the skull. These plots illustrate the magnitude of shape differences between hunter-gatherers (Indian Knoll Archaic, red diamonds), horticulturalists (Woodland, green diamonds), farmers (late prehistoric, blue diamonds), and South Amerindian farmers (Pampa Grande, open squares; Bolivians open circles). Ellipses of 90% range are presented for each group. **(A)** First (center) and second order configurations (periphery). **(B)** Third order configurations.



**Fig. 3.** Shape variation along the first two principal components in lateral, frontal, and basicranial views for the whole skull configuration shown in Figure 2A. For each principal component, a wireframe is shown for the upper and lower extremes of variation along that component in the sample. Shape variation along the first principal component corresponding to the allometry-free dataset is presented in the middle row. Arrows represent main differences among pure shape and shape after the removal of allometric component analyses.

the Woodland sites, especially in the dimensions connecting the zygomatic arch and occlusal plane with the lateral and posterior walls of the vault, respectively. In particular, size-adjusted distances between the zygomatic arch and landmarks on the posterior aspect of the cranium (euryon and opistochranium) are 13–16% greater in the hunter-gatherers; in addition, in the hunter-gatherers the distance from the maxillary tuberosity to euryon is ~15% longer. Several differences in shape are also evident across the anterior, rostral portion of the face. Notably, interlandmark distances between the lower face (e.g., the AM1 and the AM2 points, the subspinal) and the opistochranium are significantly longer (13–14%), indicating a wider and taller rostrum in the hunter-gatherers. This pattern of differences is similar to the one observed between the hunter-gatherers and the late prehistoric maize farmers (Fig. 4b), being the changes concentrated in larger dimensions among the zygomatic arch landmarks and the lateral part of the vault (distances 15–16% larger in hunter-gatherers). Conversely, the inferior rostrum is not differentiated to the same extent as in the hunter-gatherer/horticulturalist comparison. In contrast to the comparisons with hunter-gatherers, differences between horticulturalists and farmers are much lower and do not involve zygomatic, alveolar, or inferior rostral traits. In summary, the EDMA analysis suggests that significant shape differences among hunter-gatherers and horticulturalists and maize farmers are primarily concentrated in terms of a reduction in zygomatic and alveolar-border dimensions in relation to the rest of the skull, and in a greater projec-

tion of the face on the hunter-gatherers as evident from Table 5 and Figure 4.

## DISCUSSION

The results presented above are congruent with the hypothesis that the shape of craniofacial structures related to mastication, specifically those related to the attachment of the temporal muscle, the zygomatic arch, and the palate, are affected by dietary shifts that presumably included softer and/or more processed foods. These shape changes are likely responses to reductions in the magnitude and/or frequency of strains these structures experience from chewing less processed foods, which can be twice those of chewing cooked, more processed foods (Lieberman et al., 2004). Conversely, the above results (Fig. 1, Table 3) do not support the general view that transition to farming is accompanied by a general decrease in the overall robusticity (Larsen, 1995, 1997), or size (Sardi et al., 2006) of the skull, or a diminution in the facial size relative to total size (Carlson and Van Gerven, 1977).

The only reduction in size observed in Table 3 is concentrated in the temporo-mandibular joint, a configuration that reflects the area occupied by the joint itself and that also is informative of the insertion region of the temporomandibular ligament (see landmarks 36, 37, 39, and 40, Table 2) and the sphenomandibular ligament, which runs from the spine of the sphenoid bone to the lingula of the mandible (see landmark 38, anterior point on the preglenoid plane, Table 2). These ligaments

TABLE 5. EDMA shape difference matrix analysis results

Dimensions significantly larger in IKA		(HG/H)	Confidence interval	
1	<b>Euryon-MW2</b>	<b>0.159</b>	<b>0.076</b>	<b>0.264</b>
2	<b>Opisthocranion-Hormion</b>	<b>0.158</b>	<b>0.086</b>	<b>0.222</b>
3	Euryon-Zygomaxillare	0.148	0.099	0.223
4	Euryon-Jugale	0.144	0.075	0.214
5	Euryon-Pterion	0.144	0.105	0.186
6	Opisthocranion-AM1	0.140	0.061	0.228
7	Subspinale-Opisthocranion	0.135	0.071	0.204
8	Opisthocranion-AM2	0.133	0.071	0.202
9	Stephanion-Lambda	0.132	0.057	0.201
10	Opisthocranion-MW2	0.129	0.077	0.191
Dimensions significantly larger in IKA		(HG/F <sub>LP</sub> )	Confidence interval	
1	<b>Euryon-MW2</b>	<b>0.166</b>	<b>0.119</b>	<b>0.220</b>
2	<b>Euryon-Jugale</b>	<b>0.155</b>	<b>0.109</b>	<b>0.204</b>
3	<b>Euryon-Zygomaxillare</b>	<b>0.151</b>	<b>0.103</b>	<b>0.204</b>
4	<b>Stephanion-Lambda</b>	<b>0.150</b>	<b>0.100</b>	<b>0.199</b>
5	Euryon-Post. infratemp. fossa	0.142	0.092	0.191
6	Orbitale superior-Lambda	0.139	0.090	0.186
7	Euryon-Ant. preglenoid plane	0.135	0.088	0.183
8	Euryon-Orbitale	0.134	0.088	0.184
9	Euryon-Lateral glenoid fossa	0.134	0.090	0.177
10	Bregma-Lambda	0.130	0.082	0.180
Dimensions significantly larger in LP		(H/F <sub>LP</sub> )	Confidence interval	
1	Asterion-Pterion	0.087	0.034	0.153
2	Asterion-Medial glenoid fossa	0.071	0.001	0.118
3	Pterion-Porion	0.070	0.015	0.130
4	Asterion-Hormion	0.069	0.008	0.110
5	Pterion-Stylomastoid foramen	0.061	0.006	0.120
Dimensions significantly larger in W				
6	Pterion-Orbitale superior	-0.081	-0.147	-0.018
7	Bregma-Opisthocranion	-0.077	-0.142	-0.003
8	Orbitale-Hormion	-0.071	-0.140	-0.002
9	Pterion-Bregma	-0.063	-0.128	-0.008
10	Pterion-Glabella	-0.052	-0.126	-0.003

Only the 10 greatest significant differences at  $\alpha = 0.05$  are presented for each comparison among the three Ohio groups. The listed interlandmark distances match lines in Fig. 4. Bolded comparisons show differences greater than 15%.

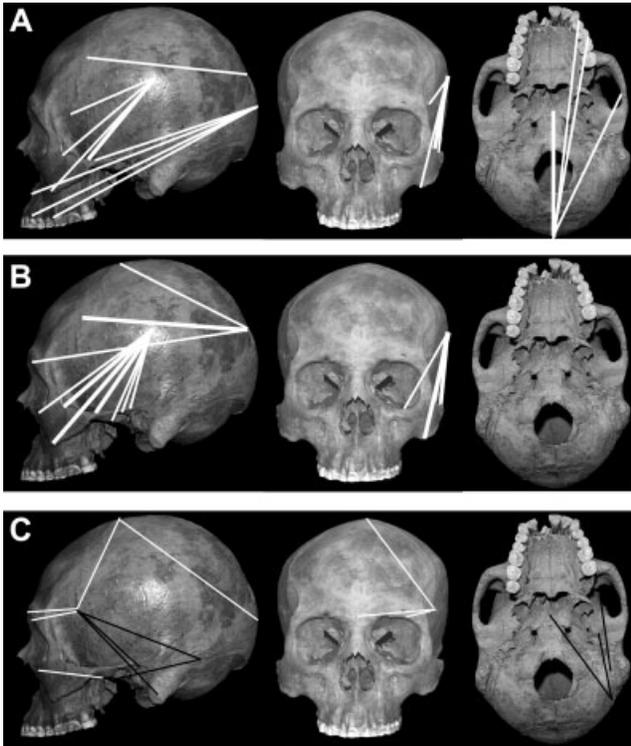
restrict movements of the mandible, and help maintain joint stability on the balancing side when high bite forces can cause TMJ distraction.

In general terms, our analyses suggest mostly localized shape changes rather than general changes involving overall size or shape. Moreover, and maybe with the exception of the alveolar component, allometric effects seem to be unimportant in these shape changes. However, comparisons among the pure and the allometry-free results should be taken with caution, since size variation in our sample is not great enough to properly study allometric effects in the context of masticatory stress. In the future, allometry effects should be addressed departing from a widest sampling including estimations of variation at the ontogenetic (within-population, across ages), evolutionary (e.g. among populations, regions, species, etc.), and static (e.g. within-population, within ages) (Klingenberg, 1998).

Experimental work on nonhuman primates focusing on the effect of diet hardness on craniofacial traits suggests that chewing hard or tough foods generates strains that are highest in the mandibular corpus, the maxilla, and the zygomatic arch (Hylander et al., 1991; Hylander and Johnson, 1992; Hylander and Ravosa, 1992; Ross and Hylander, 1996; Ravosa et al., 2000). Since humans are characterized by a highly retracted face, it is also useful to look at experimental data from nonpri-

mate mammals with more retrognathic faces, such as hyraxes (Lieberman et al., 2004). Significantly, hyraxes are characterized by a generally similar strain gradient, although perhaps with somewhat more twisting and shearing of the face. In general, hyraxes raised on cooked food had significantly less growth in the inferior and posterior parts of the face, where strains are highest. In particular, hyraxes raised on hard food developed transversely wider and longer faces primarily along the ventral aspect of the cranium, with correspondingly smaller dimensions of the dorsal portion of the rostrum and between the anterior rostrum and the posterior portions of the face. In addition, the mandibular corpus in the hard food group was significantly thicker and taller than in the soft food group (Lieberman et al., 2004).

It is interesting to note that these experimental data are partially corroborated in the human comparisons studied here, but instead of observing a broad gradient of the effect of softer diets, our results—specifically the Goodall's *F* and the PCA plots—point to an especially concentrated change in the landmark subset describing the crosssection of the temporal musculature at the level of the zygomatic arch and the origin of this muscle on the cranial vault. These results should be regarded in the context of previous studies considering the architectural complexity of the temporalis muscle (Korfage and van Eijden, 1999). Regional differences exist in length,



**Fig. 4.** Results of EDMA shape difference matrix analysis of hunter-gatherers (HG, Indian Knoll Archaic), horticulturalist (*H*, Woodland), and farmers (*F*, late prehistoric). Details of analysis are provided in the text. (A): HG versus *H*; (B) HG versus  $F_{LP}$ ; (C) *H* versus  $F_{LP}$ . Thick lines: differences greater than 15%, medium lines: differences between 10 and 15%, thin lines: differences inferior to 10%. White lines are interlandmark distances (scaled by the geometric mean of all interlandmark distances) significantly longer in HG (A and B) or *H* (C). Black lines are interlandmark distances (scaled by the geometric mean of all interlandmark distances) significantly longer in *H* (A) or  $F_{LP}$  (B, C). Interlandmark distances definitions are provided in Table 5.

spatial orientation, and position of muscle fibres, and in crosssectional area of the temporal muscle (van Eijden et al., 1996, 1997). Hence, during jaw movements fiber and sarcomere excursions are not the same for various muscle portions, and as a consequence the maximum force and excursion range of the muscle portions differ. This suggests that different portions are specialized for certain functions and that the muscle can actually exert different mechanical actions (Korfage and van Eijden, 1999). In addition, electromyographic studies have demonstrated a differential activation depending on the motor task that was executed (McMillan, 1993; Blanksma et al., 1997; Korfage and van Eijden, 1999). These studies indicated that the anterior regions of the temporal muscle are in general more intensively used than the posterior regions. The question can be raised whether the heterogeneous distribution of different muscle fiber types in the temporalis muscle, in combination with slight changes in orientation and position of the muscular attachment and crosssection, as reflected in our results, represents an architectural response to hard diets much more specific and refined than a general increase in size.

Also, the PCA analyses presented in Figure 3 suggest that some effects of softer diet could be detected in terms of the orientation of the lateral margin of the zygomatic and the shape of the palate. Other studies made on relatively retrognathic nonhuman primates like the New World monkey *Aotus* suggest that during incision and unilateral mastication, the face is subjected to upward bending in the sagittal plane resulting in rostrocaudal compression of the interorbital region (Ross and Hylander, 1996). If such strains are large in humans, then variations in diet hardness do not appear to generate shape changes in the upper face as a response to these strains (Fig. 2b).

As in many experimental studies, comparative analyses tend to simplify the environmental stimuli into overly broad nominal categories such as “hard” versus “soft” diets. It should be evident that this approach probably does not usefully categorize the entire set of strains and stimuli that the skull experiences. In this context, caution is required about formal terms such as “hard” versus “soft” to separate what is known about the true parameters (stiffness, toughness) and general technological level (hunter-gatherer, farmer, etc.). In humans, hardness of diet is usually a function of the degree of food processing. Foods whose preparation involves maceration, boiling, grinding, etc. tend to be softer and require lower magnitudes of force to comminute than unprocessed foods (Agrawal et al., 1997; Lucas, 2004). Similar differences in toughness also characterize processed versus unprocessed foods. Presumably, the Ohio valley populations chewed foods that varied and/or differed to some extent in both hardness and toughness over time. Dietary reconstructions during the Ohio valley’s technological transition suggests an increasing sophistication of food preparation techniques across the technological transition from the late Archaic (10000–3000 BP), to the Woodland (3000–1200 BP), and the late prehistoric (1200–500 BP) period (Greenlee, 2002). Archaeological evidence indicates that effort put in the processing of foods (cooking, boiling, storing) intensified after the Woodland period, as reflected by the increased frequency of pottery in general and ceramic vessels in particular (Kellar, 1983). The use of stone and ceramic tools reaches their maximum on the late prehistory phase, in parallel with the shift to extensive maize farming. Analyses of dental wear, microwear, and pathologies are fully congruent with the archaeological interpretation (Sciulli, 1997, Schmidt, 1998, 2001). For example, teeth from the late prehistory burials are characterized by a very low frequency of pits and scratches, which is a classical marker associated to the food hardness.

Nonetheless, further archaeological research is needed to test how much food cooking and/or processing different between hunter-gatherers and farmers. In addition, more research is needed on the possibility that the crania in the different populations may be adapting to differences in masticatory stress in ways that cannot be easily detected using the methods employed here. For example, thickening as well as alteration of the material properties of cortical bone could represent an alternative way to decrease masticatory stress.

Regardless of the need for more complementary data, an important question is to what extent the observed results reflect plastic changes due to the effect of diet shifts or changes that result from unknown genetic fluctuations associated with drift (Roseman and Weaver, 2007) or from population displacement or replacements.

An intuitive approach is to take a modularity scheme to the whole skull, to assume that underlying genetic differences among samples are maximized when analyzing all regions in combination (according to Relethford 1994), and then to compare the level of differentiation across the different, particular structures. The logic implicit in this approach is that if a priori genetic differences exists among samples, the effect of a given environmental effect can only increase these differences. Such a scheme was applied recently after the computation of  $F_{st}$  values across different regions (González-José et al., 2005). The authors suggest that masticatory and/or alveolar regions maximize the discrimination between economic strategies rather than between local populations, reflecting the potential plastic response of these structures to hard diets.

Here we have adopted an alternative approach, by selecting a specific sample whose diet and population history are reasonably well known and using a modularity scheme reflecting the hierarchically nested nature of morphological integration (Willmore et al., 2007). Studies based on samples reflecting a pure cultural (non genetic) transition from foraging to farming are difficult to undertake given the need for large, well-preserved sample sizes without artificial cranial deformation, and with comprehensive archaeological data on diet. Even though some of our results show between-group differences in the vault, base, or face, the majority of the differences among hunter-gatherers, horticulturalists and farmers is concentrated in the temporal fossa region, as well as a lesser degree of change in terms of the vertical orientation of the zygomatic and the shape of the palate. Obviously, these changes could have a genetic basis (e.g., from drift), but the most remarkable result here is that the significant masticatory changes across the Ohio valley sequence, presumed to present minimized genetic differences, parallel those between the two Southern Amerindian farmer populations studied.

In summary, between-group shape differences are observable in many structures, but the magnitude of change is greatest for comparisons of masticatory landmark configurations between populations presumed to differ in diet. A careful inspection of Figures 1 and 3 show that differences among hunter-gatherers and farmers cannot be reduced simply to a trend of skull gracilization. For instance, many cranial regions of different order tend to be relatively smaller on the hunter-gatherers. As noted above, the most consistent and strongest difference between groups is the relative position of the superior attachment of the temporalis muscle (stephanion), which is displaced anterosuperiorly in the hunter-gatherers sample. This suggests a relative expansion of the anterior compartment of the temporalis, which is most active in producing bite forces on the posterior dentition. Other shape differences observed above are also commensurate with a larger, wider origin of the masseter, and a broader palate. Based on these results and on the archaeological context and dietary reconstruction of the samples, it appears that these architectural differences indicate increased bite force in the hunter-gatherers, and perhaps increased mechanical advantage. An apparent paradox of our results is that the hard diet sample has smaller skulls and faces, which is counter to conventional wisdom. Thus, our data suggest that dietary shifts, at least in some populations, are not necessarily tied to a decrease in size. Conversely, our

results could indicate that some shifts in biomechanical efficiency can be concentrated on localized shape and orientation changes affecting structures directly related to mastication. An inspection of the apportionment of variation on the temporal fossa subconfiguration (Fig. 2b) also suggest that, perhaps, relaxed masticatory demands are associated with decreased levels of variation (see Wood and Lieberman, 2001). This is a suggestive result that deserves further analysis using both, experimental and comparative data. Thus, assuming that genetic differences are controlled, or at least minimized across the Indian Knoll–Woodland–late prehistoric chronological and technological sequence, our results point to a localized effect of diet quality on the shape of the temporal-zygomatic and alveolar-palatal structures, rather than on size aspects.

Our results also raise some interesting points related to morphological integration of masticatory structures. In a recent paper, Lieberman (2008) speculated that facial reduction in humans should be considered as an integrated response to a set of coselected traits such as vocalization, locomotion, mastication, cognitive abilities, and respiration. Thus, reduction of some masticatory dimensions related to softening of diet, as evidenced by the EDMA analysis (see Fig. 4), could be viewed not only in the context of masticatory strain resistance, but also in terms of other, more complex selective pressures involving a range of functions.

Interestingly, the Goodall's  $F$  test indicates that the second-order neurocranial configuration and their corresponding vault third-order subset differ strongly among groups. This result is also underlined by Figure 2b, since this figure depicts that the temporal-fossa configuration provides a great deal of discrimination among farmers and hunter-gatherers. Moreover, this result is congruent with previous finite-element analysis studies made on primate models reflecting an integrated response of the inferior rostrum and other facial structures to variations in masticatory loadings (Strait et al., 2007; Wroe et al., 2007).

The experimental-derived expectations concerning response to variations in the hardness of diet are partially confirmed in a natural recent human sample. In fact, the obtained results suggest that the effect of softening of diet on craniofacial shape is concentrated in the relative reduction of temporal fossa and in the orientation and relative position of the temporal muscle attachment. Finally, some of our results could be viewed as evidence of hierarchically inclusive integration among traits: joining muscle attachment and temporo-mandibular joint present similar patterns of diversification, and some proportion of variation in the vault could be attributed to a coordinated response with masticatory structures.

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