

Testing Hypotheses About Tinkering in the Fossil Record: the Case of the Human Skull

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ABSTRACT Efforts to test hypotheses about small-scale shifts in development (tinkering) that can only be observed in the fossil record pose many challenges. Here we use the origin of modern human craniofacial form to explore a series of analytical steps with which to propose and test evolutionary developmental hypotheses about the basic modules of evolutionary change. Using factor and geometric morphometric analyses of craniofacial variation in modern humans, fossil hominids, and chimpanzee crania, we identify several key shifts in integration (defined as patterns of covariation that result from interactions between components of a system) among units of the cranium that underlie the unique shape of the modern human cranium. The results indicate that facial retraction in modern humans is largely a product of three derived changes: a relatively longer anterior cranial base, a more flexed cranial base angle, and a relatively shorter upper face. By applying the Atchley-Hall model of morphogenesis, we show that these shifts are most likely the result of changes in epigenetic interactions between the cranial base and both the brain and the face. Changes in the size of the skeletal precursors to these regions may also have played some role. This kind of phenotype-to-genotype approach is a useful and important complement to more standard genotype-to-phenotype approaches, and may help to identify candidate genes involved in the origin of modern human craniofacial form. *J. Exp. Zool. (Mol. Dev. Evol.)* 302B:284–301, 2004. © 2004 Wiley-Liss, Inc.

Paleontologists (e.g., Gould, '77) have long sought answers to questions about the developmental bases for major transformations evident in the fossil record including speciation events such as the origin of *Homo sapiens*. Recent advances in evolutionary developmental biology (EDB) have revived interest in many of these questions but have been of limited use to paleontologists for three reasons. First, the model animals that are the focus of most developmental research (e.g., mice, zebrafish, and fruitflies) are not always good analogues for many of the smaller-scale evolutionary changes evident in the hominid fossil record such as bipedalism, increased encephalization, or canine reduction. A second problem is the lack of ontogenetic data necessary to test many developmental hypotheses. For most fossil species, we only have adult or subadult forms, hampering efforts to study even basic changes in patterns of postnatal development, let alone providing the

opportunity to glimpse any details of developmental processes during embryogenesis. Finally, comparisons of distantly-related model organisms in a phylogenetic framework are useful for studying the origin of large shifts in phenotype such as novel organs, but most shifts relevant to human evolution are instances of tinkering. Tinkering has many definitions (e.g., Jacob, 2001); here we use the term in a particular sense *as small scale shifts in development that cause changes within a body plan* such as alterations in the relative size of structures. Most microevolutionary events (e.g., speciation) occur through tinkering.

Given the inherent limitations of paleontological research, it is useful to ask how we should try

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to test hypotheses about tinkering in the fossil record. This topic is of special interest given recent advances in comparative genetics and genomics. By the time this paper is published, the complete genetic sequence of the chimpanzee should be available, providing biologists with a long list of candidate genes that differ between humans and chimpanzees, only some of which played a major role in human evolution. As Carroll (2003) has pointed out, the hard work will only just have begun because of the enormous difficulty in relating genotype to phenotype. EDB research on the processes by which genotype generates phenotype in model organisms will be critical for informing research strategies on problems in primate and human evolution. However, as with most evolutionary transformations, the phenotypic changes evident between humans and chimpanzees are unique, and will not be testable or explicable purely on the basis of simple analogies or extrapolations from model organisms. In addition, these transformations occurred in multiple stages that we must reconstruct from the fossil record of human evolution.

Of the many evolutionary events to study in hominid evolution, we focus here on one specific problem, the origin of modern human cranial form. However, the issues we review should be generally applicable to other tinkering events in the fossil record. After briefly summarizing major questions about the origin of modern human cranial form, and the data ideally necessary to test hypotheses about this transformation, we propose a framework for making some inferences in the absence of these data, and apply the approach using available data—some of which has been previously published (Lieberman et al., 2002; McBratney-Owen and Lieberman, 2003)—on craniofacial integration in humans and on mechanisms of cranial development in other organisms. We stress that this is a speculative review using a framework with inherent limitations and with insufficient data to fully test our hypotheses.

The problem: modern human craniofacial form

The morphological changes associated with the origin of modern human cranial form are subtle in comparison with the two larger-scale shifts in overall skeletal morphology that occurred earlier in human evolutionary history. The first was a shift sometime between 5 and 8 Ma from our last

common ancestor with chimpanzees—a creature that was probably very chimpanzee-like—to one or more species (candidates include *Sahelanthropus tchadensis*, *Ardipithecus ramidus*, and *Orrorin tugenensis*) that were probably bipedal, with thickly-enameled cheek teeth and small canines (White et al., '94; Senut et al., 2001; Brunet et al., 2002). The better-known descendants of these early hominids, the australopithecines, appear to be variations on the same theme: they were chimpanzee-sized (30-50 kg), with slightly larger brains; thicker, larger postcanine teeth, and smaller front teeth than chimpanzee's; and they were habitual bipeds but retained many adaptations for arboreality (for a review, see Klein, '99). The second transition was the origin of *H. erectus (ergaster)*, probably some time before 1.8 Ma. Early *H. erectus* fossils differ substantially from australopithecines in several key respects: they are considerably larger in body mass (up to 70 kg) with both absolutely and relatively larger brains (typically greater than 700 cc); they have tall, modern-shaped bodies committed to bipedalism with no evidence for arboreality (for review, see Klein, '99). Consequently, species such as *habilis* and *rudolfensis* (both of which lived prior to *H. erectus*) are more properly assigned to the genus *Australopithecus* instead of *Homo* (Wood and Collard, '99).

In contrast to the multiple changes in the skeleton that occurred with the origins of the earliest hominids and with the origins of the genus *Homo*, the evolution of “anatomically modern” *H. sapiens* (AMHS) appears to have involved fewer and less dramatic skeletal changes. Genetic, paleontological and archeological data all indicate that AMHS most likely evolved in Africa between 160 and 250 Ka (Klein, 2003; White et al., 2003) from large-brained descendants of *H. erectus*, usually classified under the general category “archaic” *Homo sp.* (AH). There is little agreement on the systematics of AH, which includes several proposed species including *H. heidelbergensis* and *H. neanderthalensis*. In part, these disagreements reflect the subtle nature of the derived morphological features that distinguish AMHS from AH. As Figure 1 illustrates, the major cranial differences between AMHS and closely related AH species are a more globular braincase, and a smaller face that is retracted underneath the anterior cranial fossa (Lieberman et al., 2002). Postcranially, we are almost identical to AH (see Pearson, 2000). Debates persist over possible cognitive and behavioral differences between



Fig. 1. Lateral view of **A**, recent “anatomically modern” *H. sapiens*; **B**, “archaic” *Homo sp.* (Broken Hill); **C**, *Pan troglodytes*. Vertical bar is line projected inferiorly from the most anterior, inferior point on the cranial base (the foramen caecum). Note that in the modern human almost none of the

face projects in front of the cranial base, whereas the face in archaic *Homo* and *Pan* is both larger relative to brain size and more anteriorly positioned relative to the cranial base. Note also the more rounded neurocranium in *H. sapiens*.

AMHS and other species of *Homo* (Klein, '95; McBrearty and Books, 2000).

Although AMHS differs from other species of *Homo* in subtle ways, there has been a tendency for researchers not only to emphasize the unique features of AMHS, but also to speculate about their adaptive value. Many paleoanthropologists have either assumed or explicitly argued that the skeletal evidence for the evolution of AMHS skull shape indicates the effects of selection on a wide variety of features related to diverse factors such as cognition, language, locomotion, and diet (for a comprehensive review, see Wolpoff, '99). According to such a view, our speciation involved multiple, independent events and selection pressures over a long period of time. However, EDB approaches challenge this view. For example, Lieberman et al. (2002) argued that many of the cranial autapomorphies used to define AMHS are an integrated suite of features whose co-appearance may result from a small number of developmental shifts that occurred early in cranial ontogeny. In particular, we found that most of the changes associated with modern human cranial form stem from an increase in neurocranial globularity and from retraction of the face, which are hypothesized to derive from interactions between brain shape and face size (see also Lieberman et al., 2000b). If the hypothesis is

correct, then many, if not most, AMHS autapomorphies may be by-products (“spandrels”) of more fundamental shifts rather than selected adaptations in their own right. However, to better infer selection pressures in human evolution, we need to test more rigorously hypotheses about the developmental bases for morphological changes evident in the fossil record.

Genotype-to-phenotype approaches

A major challenge confronting all paleontologists, especially those interested in tinkering events in the fossil record of non-model organisms, is how to make inferences and test hypotheses about the developmental causes of these events. In general, there are two types of methodological approaches (both hypothetical-deductive) to this problem: genotype-to-phenotype, and phenotype-to-genotype. The first, based on typical EDB approaches using model organisms, is to identify candidate genes that are potentially implicated in a morphological shift of interest. In the case at hand, this might involve using knock out/in experiments, transgenic expression experiments, micro-array studies, and genetic pathologies to look for mouse genes that have phenotypic effects similar to the ones in which we are interested such as facial retraction or brain size increases (for an

extensive review, see Carroll, 2003). Once candidate genes of interest have been identified, their relevance to the particular problem must be tested on a case-by-case basis. An interesting cranial example of this approach is the study of the *Br* allele which causes cranial base shortening, facial retraction, and frontonasal dysplasia (Lozanoff, '93; Ma and Lozanoff, '96). Skeletal analyses show that *Br/+* mice have a deformed anterior sphenoid, and *Br/Br* homozygotes lack an anterior sphenoid (McBratney et al., 2003). Although the *Br* gene is involved in normal cranial base development, it alone cannot account for the craniofacial changes evident in human evolution. However, it and other genes that play a role in normal anterior cranial base development (e.g., *Cart-1*) are candidates for being involved in whatever transformation occurred (see below). Genotype-to-phenotype approaches are common in EDB, but they have several limitations (reviewed in Carroll, 2003). Perhaps the biggest challenge is to sort out not only which developmental pathways were altered, but also which aspects of the pathways changed. There are many ways in which developmental pathways can be modified to generate a particular phenotype (Wilkins, 2002). Thus identifying a candidate gene with particular phenotypic effects (e.g., a patterning gene that specifies identity, or a signaling factor that regulates cell proliferation) is insufficient evidence to test the developmental bases for a phenotype's evolutionary origin.

Another, more practical problem with applying genotype-to-phenotype approaches to skeletal evolution is recognizing *a priori* the appropriate developmental pathways to study. Of the thousands of genes that differ between a human and a chimpanzee, which ones have the most important effects, and why? A better way to phrase the question is how did selection alter the developmental pathways that result in key skeletal variations? Clearly it is neither possible nor desirable to study the expression of each candidate gene in model organisms, let alone in humans and chimpanzees. Current practice is to focus opportunistically on any gene for which there is evidence of some kind of difference in expression between chimpanzees and humans. Most of these genes will turn out to be irrelevant or problematic. For example, micro-array studies have identified several genetic differences between human and chimpanzee brains (Enard et al., 2002a,b), but the role of these genes in human evolution remains unclear given the lack of any understanding of

how they actually affect phenotype (Carroll, 2003). This problem is no less true of skeletal traits which are highly integrated, polygenic structures whose morphology is the product of multiple epigenetic interactions between a diverse array of tissues and environmental stimuli at many hierarchical levels of development. Defining the appropriate units of change is a concern because the complexity of developmental pathways provides numerous opportunities for evolutionary shifts in development, and confounds the existence of discrete phenotypic traits with discrete morphogenetic bases (Wagner, 2001; Wilkins, 2002). As a hypothetical example, thickening of cranial vault bones could be caused by a gene that increased the number of GH or IGF-1 receptors in the cranial endosteum, or it could have derived from the effects of mechanical loading on the skull or some other epigenetic change (see Lieberman, '96). Given the problems of relating phenotype with genotype, how do we identify phenotypic units that reflect discrete shifts in development?

Phenotype-to-genotype approaches

A useful complement to genotype-to-phenotype approaches is to work backwards from phenotype to narrow the list of developmental processes and candidate genes to study. This approach not only has several practical merits, but is also necessary for examining evolutionary events that can only be observed directly in the fossil record. Paleontologists with little recourse to genetic data typically deal with the problems the fossil record poses by using patterns of morphological integration (defined here as patterns of covariation that result from interactions between components of a system) to first identify and/or define traits that are hypothetical developmental modules (defined here for the skeleton as morphogenetic units at the organ level, and condensations at the cellular level). Modular changes evident in the fossil record can then lead to hypotheses about their developmental bases.

The primary challenges of this approach are to avoid making simplistic assumptions about correspondences between genotype and phenotype, and to devise testable hypotheses. These challenges are evident in a recent effort by Lovejoy et al. ('99), who categorized five types of morphological traits in terms of their utility for making evolutionary inferences. These types range from traits whose expression is the direct result of genetic modules that have fitness effects upon which selection

operates, to traits with highly epigenetic developmental pathways in which non-genetic stimuli influence phenotypic variation to a large extent. The former types of traits (they suggest as an example superoinferior shortening of the ilium in hominids) might be useful for phylogenetic analyses in the sense that they are heritable, but are also potentially subject to homoplasy. The latter types of traits (they suggest as an example variations in the bicondylar angle of the femur) are probably most useful for reconstructing habitual behaviors that may be unrelated to taxonomy. This typology has merits, but is currently difficult to operationalize for three reasons. First, as noted above, there are many ways by which developmental pathways can be modified to generate a particular phenotype, making it difficult to assume that a given phenotypic variation is the result of a particular hypothesized genetic shift. Second, it is rarely possible to identify the developmental pathways by which a set of genes influences the expression of a single phenotypic trait. This is a special problem for most skeletal features, which are typically polygenic and highly integrated, blurring not only the identity of evolvable units, but also their genetic bases (e.g., Livshits et al., 2002). Third, processes of epigenesis and pleiotropy that affect a given trait may be highly evolvable (Cheverud, '96; West-Eberhard, 2003). It follows that to infer and test hypothetical modules (co-varying units) of evolutionary importance, it is first necessary to analyze changes in patterns of morphological integration during ontogeny and between ancestor and descendant species. This task is complicated by changing patterns of integration at different levels of development (which alter modular identity), and by the complexities of inferring process from pattern.

Given these problems, a four-step framework may help to propose and test preliminary hypotheses about the developmental basis for tinkering events in the fossil record between ancestor and descendant species (assuming the phylogeny is known). The first three steps focus on identifying patterns of change, and the last step focuses on identifying processes involved with those changes.

Step 1

As noted above, in the absence of good data on the relationship between genotype and phenotype, a first step involves analyzing patterns of integration in regions of the phenotype to identify or at

least hypothesize structural modules, defined here as integrated suites of features that co-vary as a result of shared development. Identifying such modules is essential for studying highly integrated aspects of the phenotype such as the skull because multiple pathways of epigenetic interaction can generate covariation among observable features that are not entirely independent. If one fails to recognize the effects of integration, then one will fail to identify useful units of analysis. There are many ways to quantify integration (for review, see Chernoff and Magwene, '99). A simple and effective set of methods is to analyze the principal components of shape variation, for example via factor analysis (Zelditch, '97) which identifies combinations of variables that account for morphometric covariation among a given sample (Sokal and Rohlf, '95; Reyment and Jörskog, '93). A factor analysis can also provide information on covariation in growth as well as shape if it is based on landmarks that are loci of growth. In a skull, such landmarks are mostly synchondroses and sutures.

Step 2

Once structural modules—hypothetical units of change—have been identified, a second step is to quantify the extent to which these modules and their resulting patterns of integration changed between taxa at comparable ontogenetic stages (typically just adults in paleontological cases). Of the many ways to quantify differences in integration between samples, including comparing correlation and covariance matrices (e.g., Ackermann and Cheverud, 2000), geometric morphometric (GM) analyses are particularly useful because they can localize significant differences in shape without reference to any *a priori* model (O'Higgins, 2000; Lele and Richtsmeier, 2001; Richtsmeier et al., 2002; Bookstein et al., 2003). GM analyses can also identify potential growth differences between samples if shape is measured using landmarks that are loci of growth.

Step 3

A third step is to test the morphological effects of hypothesized changes in patterns of integration during ontogeny. As noted by constructional morphologists, if a phenotypic module differs among adults of two species, then the ontogeny of these modules should also differ in ways that temporally or spatially reflect more proximate developmental causes of the observed differences

in pattern (Hall, 2002). For example, shifts in a given module of covarying features (e.g., braincase shape) may derive from changes among a subset of landmarks. As noted above, ontogenetic studies of this nature are almost always impossible for fossil taxa. Even for *H. neanderthalensis*, the best sampled fossil hominid species, there are no reasonably complete crania of individuals younger than two years, which is relatively late in ontogeny for neurocranial growth, as growth in the cranial base is nearly complete, and brain growth is at least 50% complete (Lieberman and McCarthy, '99). In addition, no juvenile Neanderthal fossils preserve a complete cranial base, making it difficult to study the aspects of cranial base growth discussed in this paper. An alternative is to test hypothesized effects of changes in a given module in closely related, extant species for which ontogenetic data exist. For example, apes are useful comparisons in considering the transition from AH to AMHS because they share with AH many of the same primitive features of interest, such as a more extended cranial base, and a projecting face (Fig. 1). Because the basic mechanisms of growth in these species are similar, we can expect shifts in a given structural module to have similar ontogenetic effects (see Hall, '99, p. 93-109).

Step 4

The above steps all focus on identifying patterns of change. Thus, a final step is to propose and test hypotheses about the developmental pathways (processes) responsible for the observed differences in morphology. Morphogenetic differences are broadly categorized into three types: heterotypy (the origin of new traits), heterotopy (change in the relative position of traits), or heterochrony (change in the timing of growth that alter trait size and shape). Generally, one expects the addition or loss of modular units to cause heterotypy, whereas alterations to these units and/or changes in their growth parameters (common forms of tinkering) typically cause heterochrony or heterotopy (see also Zelditch et al., 2000), whose morphogenetic bases in complex skeletal traits have previously been outlined by Atchley and Hall ('91). According to the Atchley-Hall model, mesenchymal condensations, the initial morphological units in the skeleton, differ in size and shape through modification of only a few key parameters, such as the initial number of cells, the timing of the initiation of condensation, the rates of cell division and migration, the percentage of

mitotically active cells, and the rate of cell death. In turn, bone size correlates with mesenchymal condensation size (Cottrill et al., '87; Atchley and Hall, '91; Cohen, 2000). Subsequent changes to skeletal units that influence morphogenesis occur through at least three different hierarchical levels of epigenetic interactions. (1) Primary epigenetic interactions cause the induction and differentiation of cell types within a condensation. In the skull, for example, most of the endochondral precursors to the cranial base and the intramembranous precursors to the face and cranial vault originate through induction between epithelial and mesenchymal cell types (for summary, see Sperber, 2001). Primary epigenetic events are obviously of extraordinary importance because they determine the basic, initial configuration of units upon which further epigenetic events depend. (2) Secondary epigenetic interactions occur between immediately adjacent tissues during growth, such as between muscle and bone, or between the brain and the cranial base. These interactions, mediated by various signaling factors, occur throughout growth, and are probably the mechanistic basis for most processes of integration between bones and other tissues. Viewed in this context, the functional matrix hypothesis (Moss, '97a-d) is essentially a model of secondary epigenetic interactions in the skull. (3) Finally, tertiary epigenetic interactions occur throughout ontogeny between cells within a unit and the rest of the organism (e.g., via hormones) as well as the environment. In the skeletal system, environmental stimuli imposed by mechanical loading are not trivial, but can have effects long after the end of normal somatic growth (Herring, '93).

Phenotype-to-genotype approaches, however, can only go so far. With luck, the above analytical steps should lead to a set of hypotheses about how changes in a given phenotypic module may derive from changes in particular developmental pathways. At this point, hypothesis testing must proceed in the reverse direction from genotype-to-phenotype as described above using experimental data from model organisms and comparisons of the human and chimpanzee genome.

Applying the framework to the origin of modern human craniofacial form

The phenotype-to-genotype approach outlined above has limitations, but nonetheless may be useful (at least in a heuristic sense) when applied to the problem of the origin of modern human

craniofacial form. We do this here by expanding on two previously published studies (Lieberman et al., 2002; McBratney-Owen and Lieberman, 2003), and incorporating published data on cranial morphogenesis from other species. Needless to say, more data exist for the first steps than for the latter steps.

Step 1 is to identify hypothetical structural modules, defined as integrated suites of features that co-vary as a result of shared development. This task is complicated in very integrated structures such as skulls that have high levels of covariation both within and between regions (Cheverud, '82; Olsen and Miller, '99; Marroig and Cheverud, 2001). As noted above, exploratory factor analysis is a useful method for making hypotheses about structural modules because it identifies the principal components of covariation among variables within a sample (for details see Reyment and Jöreskog, '93). As an example, we extend here the analyses presented in Lieberman et al. (2002), calculated from samples of 100 recent modern humans with diverse cranial form, 10 Pleistocene fossils attributed to "anatomically modern" *H. sapiens* (AMHS), and 9 Pleistocene fossils attributed to "archaic" *Homo sp.* (AH).

Details of the sample are summarized in Table 1. Variables measured are summarized in Table 2; the variables are the same as in Lieberman et al. (2002) with the addition of cranial base angle and anterior cranial base length (see below). Note that variables included in the analysis are not meant to describe overall cranial shape or variability (as in Howells, '73) but instead focus on a subset of craniometric variables that are thought to differentiate adult crania of AMHS from AH (Day and Stringer, '82; Stringer et al., '84; Lahr, '96). We focus on these traits rather than a larger set of variables because we are interested primarily in which *derived* aspects of human cranial form co-vary. Co-variation in the skull is so prevalent, that analyses with large number of variables (most of which are plesiomorphic) are hard to interpret within a developmental or evolutionary framework.

Table 3 presents the results of two analyses, in which factors were extracted using principal components analysis from the recent AMHS sample and from the combined recent and Pleistocene AMHS samples using Statview 5.2 (SAS Institute, Cary, NC). To avoid making assumptions about co-variation among factors,

TABLE 1. Comparative sample used in factor analysis

Fossil/population	Taxon	N	Sex	Location
Australian Aborigines	recent <i>H. sapiens</i>	20	10 male, 10 female	AMNH
China	recent <i>H. sapiens</i>	20	10 male, 10 female	AMNH
Egypt	recent <i>H. sapiens</i>	20	10 male, 10 female	PM
Italy	recent <i>H. sapiens</i>	20	10 male, 10 female	PM
Ashanti, West Africa	recent <i>H. sapiens</i>	20	10 male, 10 female	AMNH
Cro Magnon 1	early <i>H. sapiens</i>	1	M	AMNH (cast)
Jebel Irhoud 1	early <i>H. sapiens</i>	1	M?	AMNH (cast)
Liujiang	early <i>H. sapiens</i>	1	M	AMNH (cast)
Minatogawa 1	early <i>H. sapiens</i>	1	F?	AMNH (cast)
Obercassel 1	early <i>H. sapiens</i>	1	M	AMNH (cast)
Predmosti 4	early <i>H. sapiens</i>	1	M	AMNH (cast)
Qafzeh 6	early <i>H. sapiens</i>	1	M?	AMNH (cast)
Qafzeh 9	early <i>H. sapiens</i>	1	F	AMNH (cast)
Skhul V	early <i>H. sapiens</i>	1	M	PM
Zhoukoudian 101	early <i>H. sapiens</i>	1	M	PM (cast)
Gibraltar 1	<i>H. neanderthalensis</i>	1	F	AMNH (cast)
Guattari	<i>H. neanderthalensis</i>	1	M?	AMNH (cast)
La Chapelle aux Saints	<i>H. neanderthalensis</i>	1	M	AMNH (cast)
La Ferrassie 1	<i>H. neanderthalensis</i>	1	M	AMNH (cast)
Shanidar 1	<i>H. neanderthalensis</i>	1	M	AMNH (cast)
Bodo	<i>H. heidelbergensis</i>	1	M	AMNH (cast)
Dali	<i>H. heidelbergensis</i>	1	M	AMNH (cast)
Broken Hill	<i>H. heidelbergensis</i>	1	M	AMNH (cast)
Petalona	<i>H. heidelbergensis</i>	1	M	AMNH (cast)

AMNH, collection housed at the American Museum of Natural History.
PM, collection housed at the Peabody Museum, Harvard University.

TABLE 2. Measurements used in factor analysis

Measurement	Abbrev.	Definition	Source
Frontal angle	FRA	Midsagittal angle underlying frontal curvature at maximum height above the nasion-bregma chord	Howells, '73
Parietal angle	PAA	Midsagittal angle underlying parietal curvature at maximum height above the bregma-lambda chord	Howells, '73
Occipital angle	OCA	Midsagittal angle underlying occipital curvature at maximum height above the lambda-opisthion chord	Howells, '73
Vault height relative to length	VHL	basion-vertex/nasion-opisthocranion	Day and Stringer, '82
Vault width relative to height	VWH	euryon-euryon/bregma-vertex	Day and Stringer, '82
Canine fossa depth	CFD	maximum subtense between zygomaxillare and alare	Day and Stringer, '82
Browridge size	BROW	size and shape grades of supraorbital torus	Lahr, '96: 344-6
Cranial base angle	CBA1	angle between basion-sella-foramen caecum	Lieberman and McCarthy, '99
Anterior cranial base length	ACL	sella-foramen caecum	Lieberman, 2000

TABLE 3. Factor analysis of *H. sapiens* cranial autapomorphies. See Table 2 for variable definitions

Variable	Analysis 1			Analysis 2			
	Factor 1	Factor 2	Factor 3	Factor 1	Factor 2	Factor 3	Factor 4
FRA	0.077	0.712	-0.278	0.111	0.657	-0.281	-0.284
PAA	-0.668	0.076	-0.328	-0.691	-0.035	-0.382	0.004
OAA	0.652	-0.190	0.170	0.629	-0.174	0.338	-0.014
VHW	0.614	0.134	-0.331	0.593	-0.043	-0.490	0.022
VHL	0.728	-0.164	-0.224	0.656	-0.326	-0.161	-0.333
CF	0.054	0.246	0.851	0.098	0.295	0.574	0.129
BROW	0.175	0.821	0.064	0.265	0.691	-0.294	0.066
CBA1				-0.066	0.600	0.332	-0.454
ACL				0.238	0.336	-0.026	0.791
% Variance	26%	19%	16%	20%	18%	13%	12%

Boldface indicates variables with factor loadings greater than ± 0.40 .

only an orthogonal, unrotated transformation was used. Analysis 1 is based on the same variables used in Lieberman et al. (2002), which quantified previously proposed AMHS cranial autapomorphies (Day and Stringer, '82; Stringer et al., '84). Analysis 2 includes two additional variables that Lieberman et al. (2002) concluded were more proximate causes for human craniofacial form: cranial base angle (CBA1, measured as the intersection of the chords from basion-sella and sella-foramen caecum), which is approximately 15° more flexed in AMHS than AH (Lieberman et al., 2000); and anterior cranial base length (ACL, measured as the distance from sella to the foramen caecum). The two analyses yield very similar results. The first analysis identifies three factors that explain 61% of the total variance.

Factor 1 is dominated by variables that quantify the 3D globularity of the cranial vault (such as the curvature of the occipital, parietal and frontal bones); factor 2 is mostly a function of two variables that reflect projection of the upper face relative to the cranial base (frontal angle and browridge size); and factor 3 primarily reflects a single trait, the canine fossa. The second analysis identifies four factors that explain 63% of the total variance. Factor 1 is essentially the same as in the previous analysis, and comprises the same variables that quantify globularity of the cranial vault. Factor 2 remains dominated by frontal angle and browridge size but also covaries significantly with cranial base angle, a major influence on facial projection (Lieberman, 2000b). Factor 3 is mostly a function of canine fossa depth, but also covaries

inversely with coronal globularity of the vault (see Lieberman et al., 2000a for a possible explanation of this relationship). Factor 4 mostly reflects negative covariation between the cranial base angle and anterior cranial base length.

One potential complication with regard to the above analysis is the potential effect of heterogeneity within the sample in which significant shape variation between populations can lead to spurious patterns of correlation among the combined sample that are not present within the subsamples. This problem is extremely hard to address among samples that include fossils, but can be potentially addressed in future studies by examining covariation using correlation matrices computed from within-group z-transformed data. We do not think sample heterogeneity affects the above results for two reasons. First most of the variation in the modern human sample is within-rather than between-population (Lieberman et al., 2000a). Second, the results of the analyses were essentially identical when re-calculated without different populations.

In summary, the factor analyses indicate that several covarying, integrated sets of variables underlie the cranial autopomorphies of AMHS: (1) a rounder cranial vault in the sagittal, coronal and transverse planes; (2) reduced facial projection, in conjunction with a smaller browridge, a more flexed cranial base, and a longer anterior cranial base; and (3) a more pronounced canine fossa. Cranial vault globularity is apparently largely independent of the other factors. It may reflect changes in brain shape, but could also reflect interactions between the size of the brain relative to the width and length of the cranial base (for more details, see Lieberman et al., 2000a; Ross and Henneberg, '95).

The covariation between browridge size, frontal angle, cranial base angle and cranial base length evident in factors 2 and 4 is reasonably well understood. Many aspects of browridge variation in primates are structural consequences of variation in facial protraction—termed neurocranial disjunction by Weidenreich ('41)—between the orbital portion of the upper face and the anterior cranial fossa (ACF). As the face grows forward relative to the ACF after brain growth has ceased, it necessarily pulls the outer but not the inner table of the frontal bone with it, thereby decreasing frontal angle and increasing the length of the browridges (Ravosa, '91a,b; Lieberman, 2000). Retraction of the face below the ACF can occur from a combination of increased cranial base

flexion, which rotates the face below the ACF, and from elongation of the anterior cranial base relative to the size of the face, which positions more of the face below the ACF (for details, see Lieberman, 2000; Lieberman et al, 2002; McBratney-Owen and Lieberman, 2003). The developmental bases of canine fossa depth are not understood and require more analysis.

Step 2 is to quantify the extent to which these modules—and their resulting patterns of integration—changed between taxa. We focus here primarily on changes in the spatial relationships between the face and cranial base that manifest themselves in variations in facial projection. A computationally powerful and biologically informative way to quantify these differences is to compare adult AH and AMHS crania using geometric morphometric (GM) analyses calculated from biological landmarks that reflect major sites of cranial growth. Sixteen 3D landmarks, listed in Table 4, were digitized using EtDips software (www.cc.nih.gov/cip/software/etdips/) from computed tomography (CT) scans of four adult fossil crania (Bodo, Broken Hill, Gibraltar 1, and Guattari) and from four robust recent adult male *H. sapiens* crania (two from Australia, two from North America) from the National Museum of Natural History (Smithsonian Institution). All CT scans had a slice thickness of 1 mm. Because of the lack of neurocranial landmarks, this analysis focuses only on the face and cranial base, and does not examine neurocranial globularity. Landmarks were compared between taxa in two ways. First, the software package Morphometrika (www.usm.maine.edu/%7Ewalker/) was used to calculate a least squares Procrustes superimposition of the landmarks, and then graph the transformation using a Thin Plate Spline (TPS) analysis. Mean AMHS cranial form was used as the target for the warp. Second, Euclidean Distance Matrix Analyses (EDMA) of the same landmarks were performed using the software package WinEDMA (Cole, 2002) to compare the matrices of all inter-landmark distances scaled by their geometric mean (a form difference matrix). Significant differences in shape ($\alpha < 0.10$) were determined using nonparametric bootstrapping of 1000 re-samples (Lele and Richtsmeier, '95). Figure 2 presents the results of the GM analyses of the AMHS crania with two samples of AH: Neanderthals and archaic humans from Africa (often classified as *H. rhodesiensis* or *H. heidelbergensis*). As Figure 2 illustrates, the principal differences (manifested as regional deformations

TABLE 4. Landmarks used in geometric morphometric analyses

Landmark	Definition
Anterior nasal spine*	Most anterior point on maxillary body at the level of the nasal floor in midsagittal plane
Basion*	Midsagittal point on the anterior margin of the foramen magnum
Bregma	Intersection of coronal and sagittal sutures on cranial surface
Foramen caecum*	Pit on the cribriform plate between the crista galli and the endocranial wall of the frontal bone; the most anteroinferior point on the cranial base.
Glabella	Most anterior midsagittal point on the frontal bone.
Lambda	Intersection of lambdoid and sagittal sutures on cranial surface
Nasion*	Intersection of the nasal and frontal sutures.
Opisthocranium	Most posterior point on cranium from glabella
Orbitale	Most inferior point on orbital margin
Pituitary point	Anterior edge of the groove for the optic chiasma, just in front of the pituitary fossa
PM point*	Average of projected midline points of the most anterior point on the lamina of the greater wings of the sphenoid
Posterior nasal spine*	Most posterior point on the maxillary body at the level of the nasal floor at the articulation of the hard and soft palates in the midsagittal plane
Prosthion	Most anterior midline point on alveolar process (between the two upper central incisors)
Sella*	Center of the sella turcica (independent of the contours of the clinoid processes)
Sphenoidale	The most posterior, superior midline point of the planum sphenoidium
Frontex	Most inferoposterior midline point on frontal squama above glabella
Metopion	Midline point of greatest elevation between nasion and bregma

*Landmarks used in the EDMA shape difference matrix analyses.

in the TPS analysis, and significant inter-landmark distances in EDMA) are: (1) decreased facial size in AMHS, particularly superoinferior height of the face, and anteroposterior length of the upper face; (2) inferior deflection of the anterior cranial base, indicating increased cranial base flexion in AMHS; and (3) elongation of the anterior cranial base. Note that the PM points (labeled in Fig. 2) are lateral to the midline cranial base at the poles of the temporal lobes, indicating that middle cranial fossae (MCF) underlying the temporal lobes are both relatively longer and wider in AMHS than AH. Two additional differences, shortening of the posterior portion of the cranial vault, and decreased midfacial prognathism, are also evident solely between Neanderthals and AMHS (Figure 2C,D), reflecting the more derived cranial form in *H. neanderthalensis*.

These results highlight several structural causes of reduced facial projection in AMHS. First, the face is anteroposteriorly shorter and the anterior cranial base is longer relative to overall cranial size in AMHS than in AH. Because the midface grows below the anterior cranial base (the roof of the face is the floor of the anterior cranial base), a shorter face relative to anterior cranial base

length causes less projection of the face relative to the cranial base. Second, the anterior cranial base is about 15° more flexed in AMHS than in AH (Lieberman et al., 2000b). Because the face grows downward from the floor of the anterior cranial base, increased cranial base flexion rotates the entire face below the ACF, decreasing facial projection. Finally, the MCF (which houses the temporal lobes) appears to be relatively and absolutely larger in AMHS. This difference is structurally interesting not only because it lengthens the anterior cranial base as a whole (see above) but also because it positions the boundary between the back of the face and the front of the cranial base—the posterior maxillary (PM) plane—more anteriorly in AMHS. The PM plane is oriented close to 90° in all primates with the midline axis of the orbits and the floor of anterior cranial base (McCarthy and Lieberman, 2001). Thus, moving the upper boundary of the PM plane forward causes the PM plane and the face as a whole to rotate ventrally, helping to tuck the face under the anterior cranial fossa (Lieberman et al., 2000b).

Step 3 is to test the morphological effects of hypothesized changes in patterns of integration

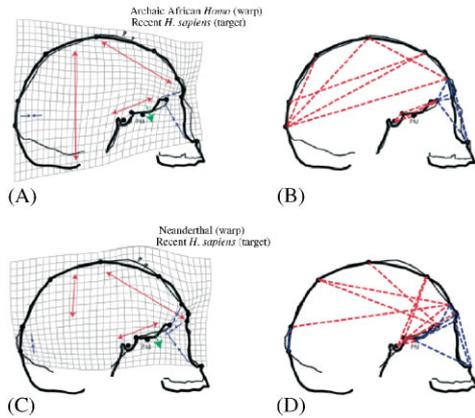


Fig. 2. Thin plate spline and EDMA form difference matrix comparisons of four robust recent AMHS crania with **A, B** two AH crania from Africa (Bodo and Broken Hill); and **C, D**, two Neanderthals (Guattari and Gibraltar). In TPS analyses to left (**A, C**), Archaic humans are the warp, modern humans are the target. Blue arrows and red arrows indicate major linear deformations in the spline that are shorter or longer, respectively, in AMHS. Dashed blue and red lines (**B, D**) are inter-landmark distances that are significantly shorter or longer, respectively, in AMHS according to EDMA form difference matrices; green arrows highlight flexion of the cranial base in the TPS. Note that the PM point (labeled) lies off the midline of the cranial base. See text for details of landmarks and methods.

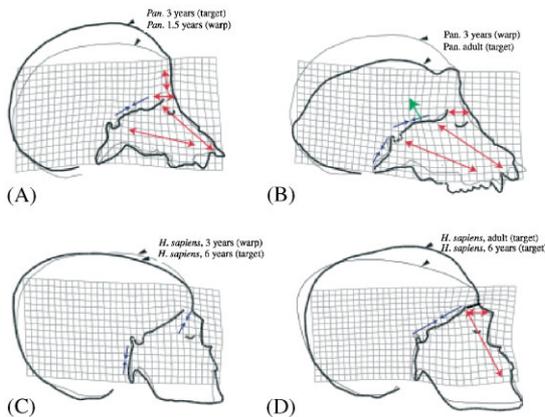


Fig. 3. Thin plate spline analyses comparing shape differences between successive ontogenetic stages in *P. troglodytes* and *H. sapiens*. **A**, *Pan* between 50% completion of neural growth period and end of neural growth period (Stages I to II); **B**, *Pan* from end of neural growth period to adulthood (Stages II to III); **C**, *Homo* between 50% completion of neural growth period and end of neural growth period (Stages I to II); **D**, *Homo* from end of neural growth period to adulthood (Stages II to III). Older stages are the targets in each comparison. Blue arrows and red arrows indicate major linear deformations in the spline that are shorter or longer, respectively, in the older ontogenetic stage. See text for details of landmarks and methods. Green arrow highlights extension of the chimpanzee cranial base in the TPS.

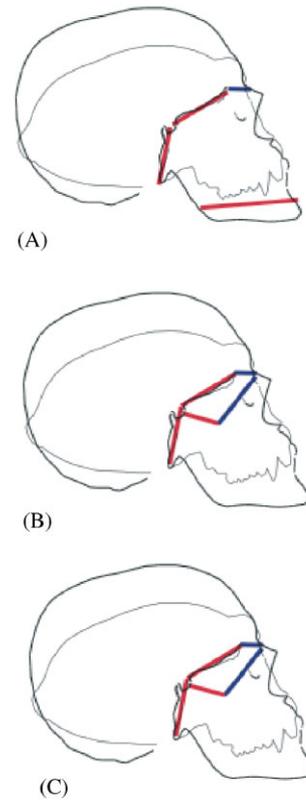


Fig. 4. Shape difference matrix summaries for comparisons between AMHS and chimpanzees. **A**, AMHS and chimpanzees at Stage I; **B**, AMHS and chimpanzees at Stage II; **C**, AMHS and chimpanzees at Stage III. Red and blue lines are inter-landmark distances that are significantly longer or shorter, respectively, in AMHS relative to chimpanzees. See text for details of landmarks and methods.

during ontogeny. As noted above, there are two ways to do this. The most direct test would be to identify the morphological units that change during the ontogeny of AMHS and AH. If the inferences made in steps 1 and 2 are correct, then the differences in proposed structural modules between AMHS and AH (facial retraction and neurocranial globularity, although we focus here on the former) should be explicable in terms of the variables identified above, namely cranial base angle, anterior cranial base length, and overall facial size.

The only fossil AH taxon for which such an analysis can currently be attempted is *H. neanderthalensis*, whose cranial ontogeny has been compared to AMHS by Minugh-Purvis ('88), Krovitz (2000, 2003), Williams et al. (2001) and Ponce de Léon and Zollikofer (2001). For the purposes of this study, all these analyses are constrained by the absence of relatively complete Neanderthals younger than two years old, and

the difficulty in gathering information on internal growth structures, especially in the cranial base. Despite these limitations, several morphogenetic shifts relevant to the above model have been identified that cause humans and Neanderthals to have different growth trajectories and cranial shape changes from the earliest stages (ca. two postnatal years). Most importantly, the human face grows less anteriorly and inferiorly from the cranial base, and the human cranial vault grows more superiorly and less posteriorly than in Neanderthals (Krovitz, 2003). However, discovery of additional Neanderthal juveniles with complete cranial bases is necessary to confirm that young AMHS crania, when compared with young Neanderthals, show increased cranial base flexion in combination with a relatively longer anterior cranial base.

In the absence of better fossil data, an alternative is to test the effects of the key variables identified above (anterior cranial base length, possibly including the MCF, cranial base flexion, and anteroposterior upper facial length), on shifts in the same structural modules using more distantly related extant species. Like AH, chimpanzees also differ from AMHS in having more projecting faces. Given the basic similarities in mechanisms of skull growth among primates (Moore and Lavelle, '74), it is reasonable to hypothesize that similar factors should underlie increased facial projection in chimpanzees and AH compared to AMHS¹. We therefore used a thin plate spline analysis and an EDMA shape difference matrix analysis to examine the ontogeny of three spatial interactions in the cranium that are predicted to influence facial retraction in AMHS and *Pan troglodytes*: (1) midfacial length relative to anterior cranial base length; (2) length of the middle cranial fossa relative to total anterior cranial base length; and (3) cranial base angle. Landmarks listed in Table 4 were digitized from lateral radiographs of a longitudinal study of six male and six female recent *H. sapiens* from the Denver Growth Study and from lateral radiographs of a cross-sectional sample of *P. troglodytes* (Lieberman and McCarthy, '99). All radiographs were of individuals at three ontogenetic stages: Stage I, 50% through the neurocranial growth phase (approximately 3 years in *H. sapiens* and 1.5

years in *P. troglodytes*); Stage II, at the end of the neurocranial growth phase (approximately 6 years in *H. sapiens* and 3 years in *P. troglodytes*); and Stage III, adult (based on third molar eruption).

The TPS analyses illustrated in Figure 3 are within species comparisons from one stage to the next that summarize changes in facial and cranial base shape. These results provide support for the hypothesis that the variables identified above influence facial retraction in AMHS (see also McBratney-Owen and Lieberman, 2003). Most obviously, the chimpanzee face and cranial base undergo more shape changes than in AMHS: facial projection increases in chimpanzees between stages I and II (1.5 and 3 years of age), as the face gets relatively longer and taller while the anterior cranial base becomes relatively shorter; during this period there is little change in cranial base angle. In contrast, the AMHS face remains retracted from Stage I to II (ages 3 to 6) during which isometry is apparently maintained between the size of the face and the size of the cranial base (Figure 3C). The major change in AMHS shape is superoinferior elongation of the face by adulthood (Figure 3D). We note that cranial base flexion in AMHS occurs during the first two postnatal years, prior to the stages analyzed here, but the chimpanzee cranial base continues to extend into adulthood (Lieberman and McCarthy, '99). This extension is likely related to the continued increase in facial projection in chimpanzees relative to the stability of facial retraction seen in AMHS from Stages I to III (Figure 3). After brain growth has ceased and before adulthood (stages II and III), both humans and chimpanzees grow mostly in terms of relative facial size, with the face lengthening mostly anteroposteriorly in chimpanzees and becoming taller superoinferiorly in humans. The EDMA shape matrix analyses in Figure 4 are between-species shape comparisons at the same developmental stage that highlight interlandmark distances that differ significantly different between species, clarifying the variables that may be associated with facial retraction. At all three stages, modern humans have relatively longer anterior and posterior cranial bases and a relatively short upper face. A relatively longer middle cranial fossa and relatively shorter midface in AMHS is first evident at Stage II (Figure 4), suggesting that a relatively long anterior cranial base and relatively short upper face are the primary influences on facial retraction prior to Stage I in AMHS (see McBratney-Owen and Lieberman, 2003).

¹Neurocranial globularity cannot be tested using chimpanzees, however, because they have less globular brain cases than both AH and AMHS; other factors such as their smaller brains, and shifts in the position and orientation of the foramen magnum, also confound comparisons of neurocranial globularity in humans and chimpanzees.

Step 4. Given the above results regarding the units of change, we can now speculate about the developmental pathways (processes) involved in the shift from AH to AMHS cranial phenotype. As described above, these shifts must involve changes in at least three variables in the craniofacial skeleton: (1) the angle of the cranial base, (2) the length of the anterior cranial base, and (3) the length of the upper face. What processes (the genes and how the genes function) cause these morphogenetic changes? Applying the Atchley-Hall ('91) model, changes in these morphological variables mostly occur via two pathways. First, variations in adult size can derive from variations in the size of the initial mesenchymal precursors through alterations of cell number, rate of cell mitosis, etc. Alternatively or additionally, variations in relative size of skeletal regions may derive from epigenetic interactions between these bones and neighboring tissues.

No data exist on the precursor size of the skeletal condensations that contribute to the cranial base and face in any primate, including humans. At this point, all we can do is hypothesize which units might be involved, and then look for genes that regulate the processes that influence the size of these units. In the anterior cranial base, the most likely units of change are the derivatives of the trabecular and hypophyseal cartilages, especially the pair of presphenoid condensations, which give rise to the anterior (prechordal) portion of the sphenoid body, including the anterior half of the sella turcica and the presphenoidal plane (de Beer, '37). The prediction is that these condensations would be significantly larger in AMHS than in AH as well as in outgroups such as chimpanzees. Most mutations in or knockouts of the genes known to affect morphogenesis of this prechordal region in mice cause hypoplasia of the presphenoid, including *Br* (Ma and Lozanoff, '96; see also Rivera-Perez et al., '95; Belo et al., '98). Interestingly, *shh* expression affects midline growth of the neural tube along with the cranial base, with overexpression causing hypertelorism and widening of the frontonasal prominence (Hu and Helms, '99). Thus, alteration of *shh* signaling may be a good candidate mechanism for changes in this region (see Ming et al., '98).

Additional and/or alternative paired units that may be involved in anterior cranial base elongation and flexion are the alisphenoids, which form the greater wings of the sphenoid around the temporal lobe; the orbitosphenoids, which form the lesser wings, surround the optic nerves, overlie

the superior orbital fissure (through which several important nerves and vessels pass), and underlie a small portion of the frontal lobe in the midline; and the mesethmoids, derivatives of the trabecular cartilages which form the front of the anterior cranial base floor (Sperber, 2001). Changes in the initial sizes of these latter condensations are less likely to cause AMHS craniofacial shape change for several reasons. First, the alisphenoids originate from the cranial base as a pair of cartilaginous anlagen, but then grow intramembranously around the temporal lobes (Scheuer and Black, 2000). It is thus reasonable to hypothesize that the temporal lobe epigenetically influences their size and shape (see below). Second, the GM analyses presented above suggest that the posterior (MCF) portion of the anterior cranial base below the temporal lobes may play a key role in anterior cranial base elongation. More research is necessary to test this hypothesis, and to distinguish between the potential effects of the frontal versus temporal lobes on this portion of the cranial base.

As noted above, another major unit to change in AMHS is the length of the face. Decreased facial length could derive from smaller condensations of the many skeletal precursors to the face, but may also occur from subsequent epigenetic interactions during growth that cause facial diminution. Further research is needed, but several lines of evidence support the hypothesis that reduced overall rates of facial growth during ontogeny might influence facial diminution in AMHS. First, the face in individuals with growth hormone deficiency show proportionately less growth in all linear dimensions except for those that derive from the cranial base (Kjellberg et al., 2000). Thus decreases in facial size of the kind evident in human evolution could possibly result from proportionately less facial growth. Second, comparisons of growth differences between juvenile AMHS and AH (Neanderthals) show almost no significant differences in overall juvenile facial growth rates (Williams et al., 2001); although by two postnatal years, the face is already relatively taller and less prognathic in AMHS than in AH (Krovitz, 2000, 2003; Ponce de Léon and Zollikofer, 2001). There are many potential mechanisms for facial diminution including modifications to the GH-IGFI axis (e.g., decreased receptor density), and the TH axis (Johnson and Bronsky, '95).

Secondary epigenetic interactions are also likely to play a role in some or possibly all of the unique aspects of AMHS cranial base growth. Recall that the cranial base is sandwiched between the brain

and the face, functioning to a large extent to integrate growth among these units, both of which influence cranial base growth (Lieberman et al., 2000b). Brain growth has potent effects on neurocranial growth via the intervening dura mater membrane, which induces osteogenesis in cranial vault sutures via expression of Fgf2 and TGF β -1 (Yu et al., '97; Wilkie and Morriss-Kay, 2001; Spector et al., 2002), and there is some reason to suspect that these pathways also act on the synchondroses. Expression of these signaling factors is neurally activated, as demonstrated by a 180° reorientation of an embryonic chick brain causing a 180° reorientation of cranial vault and base morphogenesis (Schowing, '68). In many cases, tensile forces generated by expansion of the brain stimulate transcription factor expression by the dura that induce growth of the vault (for review, see Opperman, 2000). Thus genes such as FGFR modulate but do not stimulate neurocranial growth. While it is generally agreed that the contents of the neurocranium have an enormous influence on its shape (de Beer, '37), less is known about the effects of brain size and shape on the cranial base. One especially important question is the extent to which the brain induces flexion of the cranial base, which as noted above is approximately 15° more flexed in AMHS than in AH. Although cranial base flexion in primates is significantly correlated with neocortical volume relative to basicranial length (Lieberman et al., 2000b), brain volume alone cannot account for flexion in the AMHS cranial base given the similar size of the AMHS and AH brain. One possibility is that AMHS cranial base flexion may be driven by the relative size of the frontal or temporal lobes. A first step in testing this hypothesis is to relate dimensions of the middle and anterior cranial fossae to the volumes of the temporal and frontal lobes, respectively. However, it is interesting to note that Lieberman et al. (2002) found the MCF below the temporal lobes to be significantly longer and wider in AMHS than in AH. In addition, the neocortex of the temporal lobe might be larger relative to hemisphere volume in humans compared to other apes (Semendeferi and Damasio, 2000; Rilling and Seligman, 2002). We stress that this hypothesis requires further testing from other sources of data, but an interesting candidate gene to examine is *C21orf5* whose differential expression in mice has been shown to influence temporal lobe volume (Lopes et al., 2003).

Additionally, or alternatively the differences in cranial base flexion between AMHS and AH may

be influenced epigenetically by facial growth. In particular, smaller faces in AMHS may contribute to more flexed (less extended) cranial bases. Prenatally, the human cranial base first flexes rapidly, and then extends (retroflexes) about 9° during the second trimester even while the brain is growing (Jeffrey and Spoor, 2002). There is some circumstantial evidence that a proportion of prenatal cranial base angulation may be due to effects of facial growth on synchondroses through mechanisms similar to those described above for the brain. Indeed, the human cranial base flexes rapidly during the first two postnatal years as the brain grows much more rapidly than the face (Lieberman and McCarthy, '99); in contrast, the chimpanzee cranial base remains stable during early postnatal life as both the face and the brain grow rapidly, but then extends approximately 14° until adulthood as the face grows forward after the cessation of neural growth (Lieberman and McCarthy, '99). Variations in cranial base flexion in patients with facial dysmorphology (Crouzon, Apert and Pfiffer syndromes) also support the hypothesis that facial growth influences cranial base angulation (Burdi, 2002), but the extent to which reduced facial growth in AMHS versus AH affects cranial base flexion requires further study.

CONCLUSION

Testing EDB hypotheses about tinkering events in the hominid fossil record is challenging but certain to become increasingly more common in the coming years, driven largely by comparisons of the chimpanzee and human genomes, and by better understanding of gene regulation in model organisms. For good reason, many of these studies will use a genotype-to-phenotype approach. However, this research will benefit from integration within a framework that also looks at the problem in reverse, from phenotype-to-genotype. By default, most EDB questions posed by the fossil record begin with questions about phenotype. In addition, working backwards from phenotypic pattern to infer which developmental processes to study provides several insights about tinkering events, as demonstrated by the above consideration of the origin of AMHS cranial form.

In particular, multivariate analyses indicate that most of the major craniofacial autapomorphies that distinguish AMHS from AH appear to reflect two integrated suites of changes: a more globular braincase (although this feature is not discussed extensively in this paper), and retraction

of the face below the anterior cranial fossa. Facial retraction, in turn, is a function of a more flexed and longer anterior cranial base, and a shorter upper face. Ontogenetic comparisons of AMHS, AH and chimpanzees suggest that these changes occur via morphogenetic shifts early in development, many of which are probably prenatal. We do not yet know which genes and developmental processes are responsible, in part because we cannot determine if the shifts we observe originate from changes in the mesenchymal condensations of the cranial base and face, from later morphogenetic processes during chondrogenesis and osteogenesis, or from epigenetic interactions between units of the skull and neighboring tissues such as the brain and the face. While genes that regulate formation of anterior cranial base precursors (notably the presphenoid and/or the alisphenoid) may be involved, several lines of evidence suggest that epigenetic interactions, especially between the brain and cranial base, or between the face and the cranial base, are more likely explanations. Thus, the most promising candidate genes to focus on are those that influence the relative size of the temporal or frontal lobes (e.g., *C21orf5*), and those that influence rates of facial growth. While changes in the size of precursor tissues (e.g., skeletal condensations) may be causal factors of evolutionary change in hominids, as evident in the murine mandible (Vogl et al., '94; Miyake et al., '97; MacDonald and Hall, 2001), secondary epigenetic shifts that regulate regional growth of cranial components may also be important. Interestingly, similar patterns of neural and facial diminution have evolved independently in many domesticated mammalian species (sheep, dogs, foxes) through artificial selection (see Leach, 2003). In these cases, artificial selection was probably not for smaller brains or faces *per se*, but for other aspects of phenotype such as docility via hormones that also affect cranial growth (Price, '99).

More research is necessary, but one implication of the above results is that the origin of AMHS cranial form did not require many independent changes, but instead occurred through a few ontogenetically early shifts in craniofacial development. In such respects, the tinkering events that were part of our speciation may not differ substantially in terms of process (but not scale or pattern) from larger scale evolutionary events that involved more substantial changes in body plan (Wilkins, 2002). Identifying these modular alterations narrows down the array of potential candi-

date genes responsible for shifts in craniofacial integration implicated in the evolution of AMHS cranial form. Obviously, many genes were involved in the origin of human craniofacial form. But, based on the results of this analysis, our hypothesis is that these genes were responsible for a limited number of autapomorphic shifts: one (possibly in the brain) that somehow caused a longer, more flexed anterior cranial base; another that caused overall diminution of facial size; and a third that involved increased globularity of the neurocranium (not discussed in this paper). The genes involved with the development of these features remain unidentified. However, the plausibility of the hypothesis is demonstrated by the *Br* allele in which a single genetic shift causes a growth defect in the anterior sphenoid that subsequently causes a variety of morphological effects similar in kind to those evident between AMHS and AH (Ma and Lozanoff, '96; McBratney et al., 2003).

Finally, the above results have implications beyond the immediate question of AMHS origins that are broadly relevant to the general problem of testing hypotheses about tinkering in non-model organisms in the fossil record. It is both necessary and profitable to integrate paleontological analyses and neontological studies of development in model organisms. Phenotype-to-genotype approaches complement genotype-to-phenotype approaches because they avoid making too many *a priori* assumptions about gene function in particular developmental pathways. Additionally, phenotype-to-genotype analyses are, by definition, necessary in paleontology, where all analyses begin with a study of morphology preserved in fossils. While it is tempting to apply EDB to paleontological problems by identifying candidate genes that could have particular phenotypic effects evident in the fossil record, such an approach has only limited utility given the complexity of developmental pathways in which changes can occur on so many hierarchical levels. As noted by Carroll (2003), there are possibly as many as 70,000 adaptive substitutions that may have played some role in human evolution, making it difficult to determine which genetic novelties played any key role in human evolution. We therefore advocate as a complement to genome-based studies an approach in which one first identifies integrated units of phenotypic change, before hunting the specific developmental pathways responsible. Of the many evolutionary problems to benefit from this approach, the developmental bases of human

origins stands out as an excellent test case for reasons other than intense interest in our evolutionary history. Although humans (and closely related extant species) can never serve as model animals for laboratory research on development, the completely sequenced genome of humans and chimpanzees, a wealth of data on genetic and developmental variation, and a fantastic, well-sampled and well-dated fossil record, uniquely complemented by an archaeological record, make human evolution a great subject for EDB research.

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