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Mouse models and the evolutionary developmental biology of the skull

Benedikt Hallgrímsson^{1,*} and Daniel E. Lieberman[†]

*Department of Cell Biology and Anatomy and the McCaig Bone and Joint Institute, University of Calgary, Calgary, AB T2N 4N1, Canada; [†]Department of Anthropology and Department of Organismic & Evolutionary Biology, Harvard University, 11 Divinity Avenue, Cambridge MA, 02138, USA

Synopsis Understanding development is relevant to understanding evolution because developmental processes structure the expression of phenotypic variation upon which natural selection acts. Advances in developmental biology are fueling a new synthesis of developmental and evolutionary biology, but it remains unclear how to use developmental information that largely derives from a few model organisms to test hypotheses about the evolutionary developmental biology of taxa such as humans and other primates that have not been or are not amenable to direct study through experimental developmental biology. In this article, we discuss how and when model organisms like mice are useful for studying the evolutionary developmental biology of even rather distantly related and morphologically different groups like primates. A productive approach is to focus on processes that are likely to play key roles in producing evolutionarily significant phenotypic variation across a large phylogenetic range. We illustrate this approach by applying the analysis of craniofacial variation in mouse mutant models to primate and human evolution.

Introduction

Intensive research on select model organisms has contributed to an explosion of knowledge in experimental developmental biology. Much new information about development, physiology, and disease has come from focusing on particular models, the systematic collection of molecular and phenotypic data about those models, the sharing of data through informatics resources, and the increasing use of high-throughput methods. Of these models, the mouse occupies a special place in terms of what we have learned about the cellular and molecular machinery of mammalian development, even though, in many ways, the mouse is an atypical mammalian species. For example, compared to humans, laboratory mice have reduced genetic variation, very short generation times, unusually high fertility, specialized diets, no deciduous teeth, and so on. An unfortunate side effect of our success with mouse developmental biology is that we've come to view developmental mechanisms through a limited and sometimes peculiar lens (Hall 1999). This problem is being addressed to some extent by increasing the number of model organisms (Davis 2004; Jenner and Wills 2007) such as zebrafish (Eiken et al. 1987) or the three-spine stickleback (Peichel et al. 2001). For both practical,

and in some cases ethical, reasons, however, many interesting species cannot be developed as models to the same extent as can mice or zebrafish. This constraint is particularly true of primates. Therefore, for evolutionary and comparative biologists interested in explaining diversity in general, and human evolution in particular, it remains important to consider carefully how can we apply knowledge based on a limited number of model species to the evolutionary developmental biology of humans and other such species. Here, we focus on a narrower version of this question: how can we apply information from mouse developmental biology to the evolutionary developmental biology of the primate craniofacial complex?

At its heart, the emerging field of evolutionary developmental biology concerns the ways in which development is relevant to evolutionary explanation (Gould 1977; Hall 1992; Raff 2000). A central concern are the developmental determinants of evolvability (Hendrikse et al. 2007) notably the role that constraints bias the generation of variation, and how developmental processes modulate the magnitude of phenotypic variation. In this sense, mice are excellent evolutionary developmental models to study these phenomena with regard to craniofacial morphology (Hallgrímsson et al. 2007a, Willmore et al. 2006;

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¹E-mail bhallgri@ucalgary.ca

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Hallgrímsson et al. 2007a; Willmore et al. 2007). For example, developmental integration among phenotypic characters is a common source of bias in the generation of variation. While such correlations may arise through selection (Cheverud 1996), once established they may influence subsequent evolutionary change. Thus correlations among traits produced by developmental interactions or shared developmental processes can produce evolutionary change in one character as a secondary effect of selection on another.

Another way to think about this problem is to ask what assumptions must one make about mouse developmental biology in order to apply the results to other species? At a simple level, for mice to be useful for understanding the developmental basis for evolvability in primates or other distantly related mammalian groups, the developmental determinants of constraints, bias, and modulation of variation must be similar. We have previously shown that, although the covariation structure of the mouse and primate cranium are quite different, there are also substantial and fundamental similarities (Hallgrímsson et al. 2004). This leads to the following arguments. First, a focus on the role of central developmental processes is a useful simplifying paradigm for applying the developmental biology of model organisms like mice to other species of mammals. Second, model organisms such as mice are essential for determining the developmental basis for phenotypic covariation structure. Finally, for some aspects of phenotype, mice are extremely useful for testing hypotheses about patterns and processes of integration that may have played important roles in primate evolution. A similar argument could be made about the role of model organisms in studying the modulation of phenotypic variance by developmental processes (canalization and developmental stability). Here, we limit the discussion to the study of the developmental determinants and evolutionary implications of constraints or bias in the generation of variation.

The “middle-out” approach

Most efforts to relate genotype to phenotype via development rely on “top-down” or “bottom-up” approaches that seek to reconstruct all of the developmental steps that link genetic variation to phenotypic outcomes (for reviews, see Gilbert and Sarkar 2000; Lieberman et al. 2004). In the former approach, one typically identifies a phenotype of interest, then searches for the specific candidate genetic causes of that phenotype and then tests how the specific genetic cause alters developmental processes to produce the phenotypic variation of interest.

This approach proceeds from altered phenotype to process perturbation to mutation. Alternatively, one can induce known genetic perturbations through transgenic models and compare the resulting phenotype with predictions based on hypotheses about gene function. In contrast, approaches, sometimes rooted in systems biology, often characterized as “top-down,” seek to understand properties or behavior of complex systems such as development before all of the mediating causal steps are known. The usual approach is to use models to generate predictions about the influence of known factors on the systems of interest. Top-down approaches can also be applied to highly integrated complexes of phenotypic variation by using data-reduction methods such as principal components analysis to narrow down the range of hypotheses to test when trying to understand the genetic and developmental origins of particular phenotypes (Lieberman et al. 2004).

Bottom-up approaches have been the basis for the incredible growth of knowledge in developmental biology in recent decades. However, unifying hypotheses and simplifying concepts remain very difficult to extract from the vast and rapidly accumulating data in developmental genetics. Quantitative modeling of developmental systems is a promising source of eventual solutions, but this field is very young. In vertebrate development, the most successful examples of predictive systems-level models of development are the models of tooth development (Salazar-Ciudad and Jernvall 2002). For most systems, though, we are far from knowing enough to model the developmental process from the genetic level upward.

Here, we suggest a simplifying approach that is an alternative to hypotheses that focus primarily on genotype-phenotype relationships. Notably, we suggest that a useful way to model the developmental bases for evolvability using model organisms, such as mice, is to consider how developmental processes structure phenotypic variations of particular interest. Questions about the developmental-genetic network that underpins those processes are related but separate from this level of analysis. Our logic is as follows: for many developmental systems, one can identify developmental processes that are particularly important determinants of some type of phenotypic variation. Such processes may be influenced by many developmental-genetic pathways and often by a vast number of potential mutations (Fig. 1). In general, a vast array of genetic variation is “funneled” to a smaller set of pathways, which in turn influence a smaller set of developmental processes. For example, the growth of the brain within the skull is a major determinant of craniofacial shape (Moss and Young

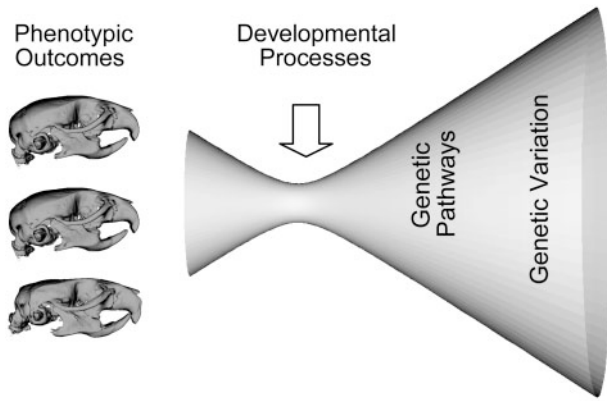


Fig. 1 A metaphorical representation of the “middle-out” approach to development. The funnel represents the potential for variation in the system at different levels of the system. The number of genetic variants greatly exceeds the number of pathways, which in turn feed into a more limited set of developmental processes. These processes then vary and interact to produce variation in phenotypic outcomes. The “middle-out” approach directs hypotheses at relationships between developmental processes and phenotypic outcomes.

1960; Enlow 1990; Moss 1997a, 1997b, 1997c, 1997d; Lieberman et al. 2000a). In turn, brain growth is a highly complex set of processes involving several cell types that are surely influenced via a large number of developmental pathways. How the brain grows is obviously relevant to brain function, but for many aspects of skull shape the most relevant variables are the timing, rate and amount of total growth within the neurocranium. The effect on skull shape is the same whether brain size increases due to generalized hyperplasia or due to hyperplasia of a glial cell type. Put differently, brain growth is a gross developmental process that can serve as the basis of hypothesis generation about the mid-level determinants of variation in craniofacial shape. Thus, to explore the effect of brain growth on craniofacial shape, a productive approach would be to compare the effects of a variety of mutations that influence the timing, rate and amount of brain growth of different degrees on craniofacial shape. Once understood, relationships between developmental processes and axes of phenotypic variation become simplifying concepts around which the vast data on specific gene effects on craniofacial shape can be organized.

Note that a central problem with the use of model organisms in evolutionary developmental biology is that the exact developmental-genetic basis for evolutionary change is probably rarely, if ever, replicated in evolution. A famous example is the selection experiment for increased tail length in mice in which different selection lines developed longer tails by very different developmental means (Rutledge et al. 1974;

Cheverud 2007). For most complex developmental systems, the potential for generating genetic variation in developmental pathways is so vast that contingency probably plays a large role in determining what particular mutations, or even genes, end up producing change along some axis of phenotypic change. The evolutionary sequences we seek to explain are thus almost always unique and unrepeatable (Williams 1966). This means that determining which genetic changes are produced by selection for some behavioral or morphological trait in mice would be very unlikely to tell us what specific genes produced change in that trait during the evolutionary history of other mammalian groups. However, inferences from model organisms become more valid at higher levels of organization and abstraction about developmental systems. For example, it is much more likely that the ways in which major developmental processes generate phenotypic variability in mice is similar to the role that such processes have played in the evolution of other mammalian taxa of interest. In the last section of this article, we show how this reasoning appears to hold for the relationship between brain growth, cartilage growth, and basicranial angle in mice and primates.

Model organisms and the developmental basis for covariation

Covariation among phenotypic traits arises when developmental processes influence particular traits or structures and not others (Zelditch and Carmichael 1989; Zelditch et al. 1993; Hallgrímsson et al. 2007b). For instance, covariation among structures in the head derived from the neural crest is due to factors that influence the spatiotemporal pattern of migration, proliferation, and apoptosis of neural-crest-derived mesenchyme while influencing other structures in the head not at all or differently. Some of these factors are intrinsic to the neural crest while others involve interactions with surrounding tissues or cell populations. Importantly, these factors must vary in order for neural crest development to generate covariation among structures derived from the neural crest. If all individuals in a population are genetically identical and experience identical environmental effects on neural crest development, then that population will not exhibit covariation among neural-crest-derived structures. Conversely, a population in which those factors vary enormously among individuals will exhibit a high degree of covariation among neural-crest-derived structures. In both cases, the developmental interactions that generate covariation are the same. The difference lies only in the degree to which the determinants of covariation exhibit variation.

In other words, covariation structure is determined by the variance of covariation-generating developmental processes (Hallgrímsson et al. 2007b; Mitteroecker and Bookstein, 2007). Phenotypic covariation structure, therefore, is dependent not just on the developmental effects of the processes that determine covariation but also on the magnitudes of the variances of all the processes (relative to each other) that contribute to the formation of that phenotype. The covariation structure of a complex phenotype is thus produced by an overlay of sequential and interacting developmental processes which, much like the layers of writing in a palimpsest, each leave their imprint on the eventual phenotypic covariation structure.

The actual developmental effects of processes can also vary, of course. For example, a growth factor may produce a pattern of pleiotropic effects by affecting several different tissues. The responses of these specific tissues to the growth factor will vary among individuals and these variances can also change through canalizing selection. Such variation in pleiotropic effects is necessary for covariation structure to evolve significantly. However, this fact is not inconsistent with the proposition that variation in covariation structure among populations or among related species is largely driven by changes to the variances of covariance generating developmental processes.

Many processes may influence the covariation structure in the mammalian skull (Hallgrímsson et al. 2007b). The processes listed in Fig. 2 are an oversimplification and likely contribute to cranial covariation structure to very different degrees. It should be apparent, however, that the covariation structure of the skull is likely to be quite complex, given so many potential covariation-generating developmental processes.

An important implication of the “palimpsest” model for the developmental basis for phenotypic integration is that the phenotypic covariance structures of natural populations cannot be used to reveal the underlying developmental factors that produce them. Weak covariation among traits does not necessarily imply an absence of significant shared developmental influences among traits. Instead, this may simply reflect the extent to which those shared developmental influences vary in relation to other processes. No matter how important a shared developmental factor may be for generating a set of traits, a lack of variation will not produce any statistical association among traits. Similarly, strong correlations among a given set of traits can arise simply by increasing the variance of developmental process that influences those traits, rather than others. Finally, covariance generating processes can “overwrite” each other if they generate opposing effects on

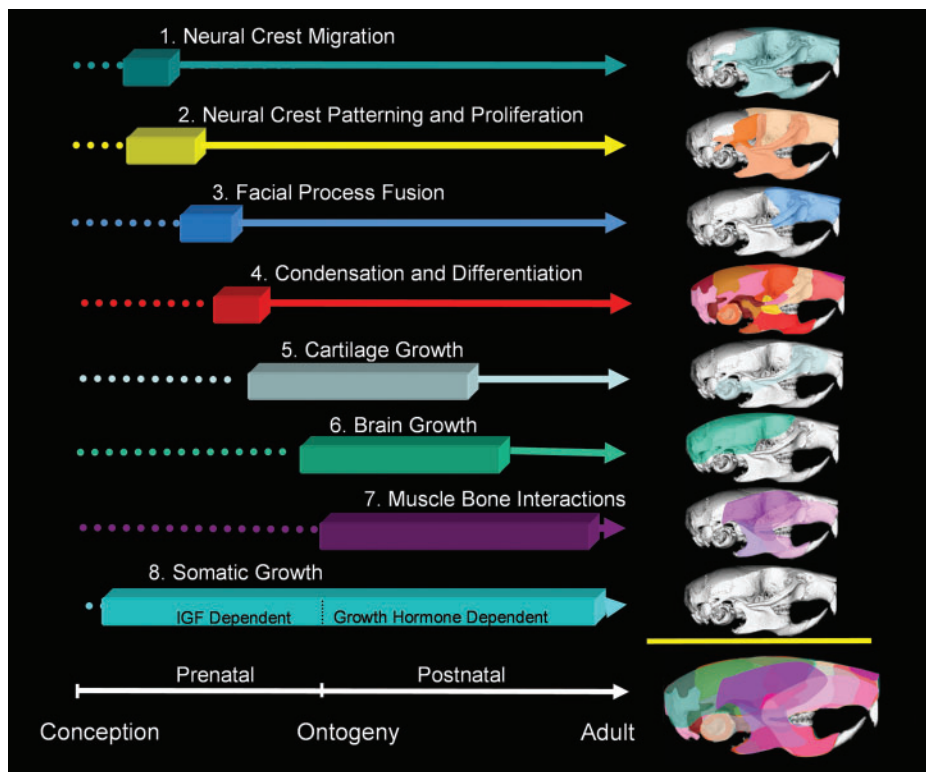


Fig. 2 Schematic overview of the major developmental processes that generate covariance in the mammalian skull.

a set of structures. Such effects should be evident regardless of whether one measures covariation as covariances or as correlations. When the covariation between traits is influenced by several processes, alteration to the variance of one of those processes relative to the others would influence the association between the traits whether measured by covariance or by correlation. Covariation structure can thus evolve through changes to the relative variance profile of the covariation generating developmental processes that contribute to a set of traits (Fig. 3). Phenotypic covariation structures thus do not necessarily faithfully reflect the developmental interactions that produce them.

For this reason, model organisms like mice are not only useful but also essential for studying the developmental factors that produce covariation. In mice, genetic variance can be minimized through the use of inbred strains so that one can isolate the influence of a specific mutation or environmental effect on covariation structure. For this reason, the developmental determinants of covariation structure can be tested by seeing how the covariation structure of a sample of inbred mice differs from the covariation structure of another such sample that differs only in some perturbation of interest. If the process influenced by the mutation or environmental factor of interest is known, then it may also be possible to measure the variance of the process rather directly through quantitative assessment of, for example, cell proliferation or apoptosis, the timing of some event like cell migration, or gene expression through RT-PCR. In natural populations, such factors can be reliably inferred rarely from phenotypic covariation structure.

Integration between the basicranium and the brain: applying mouse models to human evolution

Primates, in general; and humans, in particular—have unusually flexed cranial bases compared to most other mammals (Fig. 4). Flexion of the cranial base changes the spatial relationship between the braincase and face such that in humans, the most extreme example of flexion, the face is rotated ventrally to the frontal lobes of the brain. This relationship between the brain, the cranial base, and the form of the face has been the subject of much speculation by biological anthropologists interested in explaining the unusual shape of the human skull. The spatial packing model, developed in detail by Biegert (1963), maintains that the angle of the cranial base changes in response to the size of the brain relative to the basicranium or relative encephalization (IRE). During the course of human evolution, the model holds, the brain became larger as the basicranium became smaller, resulting in a more domed neurocranium and a more highly flexed basicranium. This model has been tested on a comparative sample of primates (Ross and Ravosa 1993; Lieberman et al. 2000b) as well as on hominid fossil skulls (Ross and Henneberg 1995; Spoor 1997; McCarthy 2001; Strait 2001). These studies generally yield comparative results that are consistent with the model. There is a correlation between brain size relative both to length and angle of the cranial base, which explains about 40% of the interspecific variation in angle of the cranial base among primates (Ross and Ravosa 1993; Lieberman et al. 2000b).

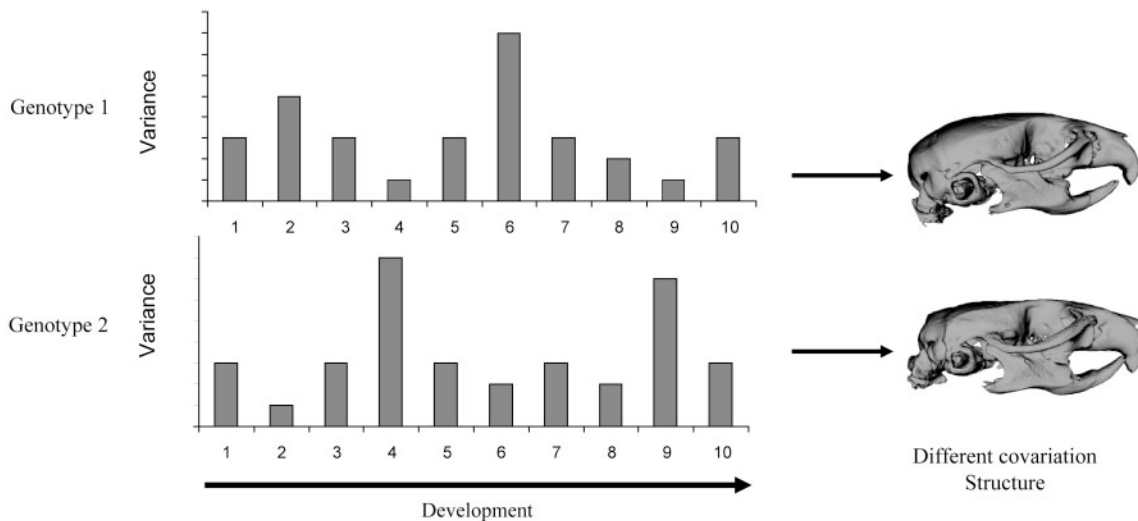


Fig. 3 The relationship between the magnitudes of the variances of covariance-generating developmental processes and covariation structure as hypothesized by the palimpsest model.

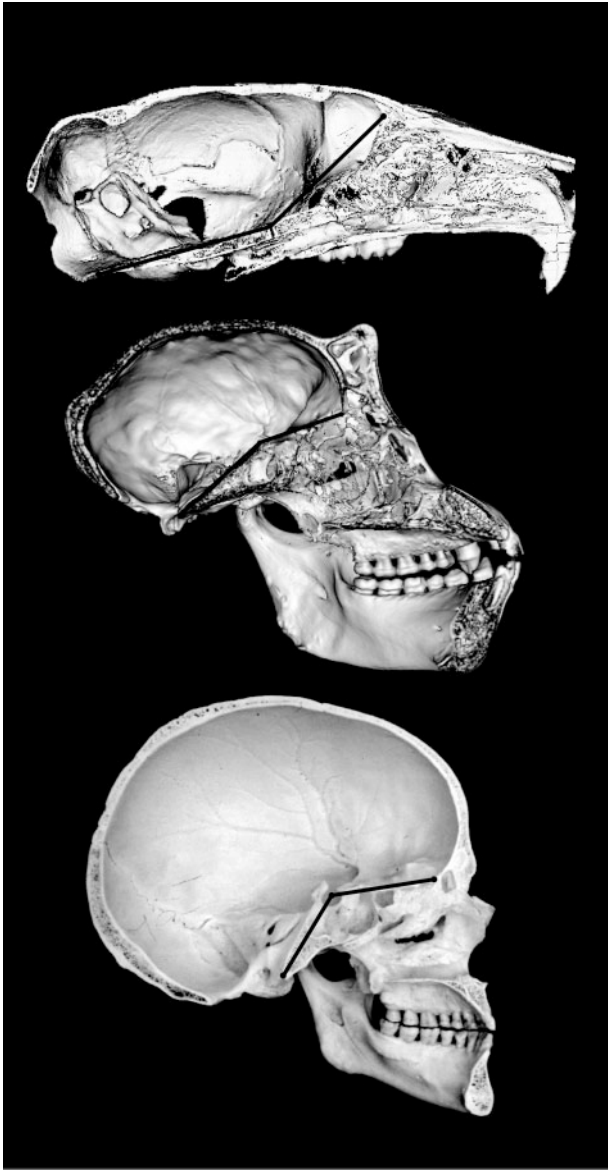


Fig. 4 The angle of the cranial base in mice, chimpanzees, and humans.

At the heart of the spatial-packing model is a hypothesis about developmentally based covariation. The model has important evolutionary significance because it implies that the position of the face evolves not as an independent trait that needs a separate functional explanation but rather, in part, as a secondary consequence of evolutionary changes in brain size. The mechanistic hypothesis is that the growing brain flexes the basicranium via tissue interactions and/or mechanical interactions so that larger brains on smaller cranial bases flex the cranial base to a greater extent than do smaller brains on larger cranial bases. Spatial packing therefore assumes some sort of epigenetically based morphological integration. This hypothesis and its underlying assumption

can be tested in mice as long as the interaction of the brain and cranial base behaves similarly in mice as in primates. That is, in both mice and primates, altering the size of the brain relative to the cranial base should produce changes in angle of the cranial base that are consistent with the model. It should be noted, however, that mice differ from primates in some craniofacial features. The cranial base in mice, for instance, is actually retroflexed (Fig. 4), and the sphenothmoidal synchondrosis in mice fuses early during postnatal growth. Despite these differences, the brain and cranial base should be similarly integrated in mice as in primates unless there is some unknown counteracting factor.

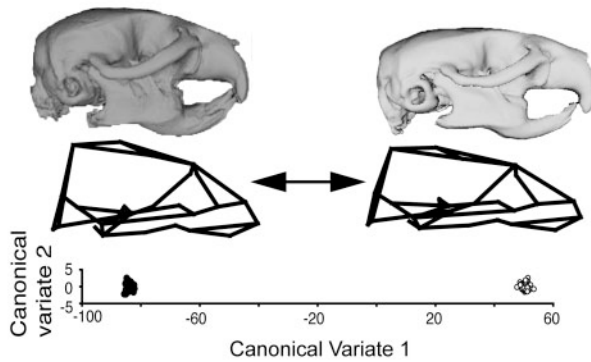
We have recently tested the spatial-packing model in a sample of mouse mutants, some of which influence craniofacial morphology in known ways (Lieberman et al. 2008). Two of these mutant strains are particularly relevant to the issue of covariation. One is the *mceph* mutation, an 11 bp deletion in the *Kcna1* gene (Diez et al. 2003; Petersson et al. 2003). *Mceph* (megencephaly) mutants (mixed C57BL/6J*Balbc/ByJ background, the Jackson Laboratory) have 25% expanded but normally shaped brains from generalized neural cell hypertrophy. The second is the Brachymorph (*BM*) mutation. *BM* mutants (C57BL/6J background, the Jackson Laboratory) have a short cranial base from an autosomal recessive mutation in the phospho-adenosine-phosphosulfate synthetase 2 gene (*Papps2*) that reduces chondrocranial growth via undersulfation of glycosaminoglycans in cartilage matrix (ul Haque et al. 1998; Kurima et al. 1998). In both cases, the size of the brain relative to the cranial base is increased. In the first case, this is due to increased brain size while in the latter it is due to reduced chondrocranial (and hence basicranial) growth. A prediction of the spatial packing model, therefore, is that the cranial base of both mutants will be less retroflexed when compared to wild type (or heterozygous) littermates.

We obtained adult (90 days) samples of both mutants as well as controls and obtained 3D landmarks for geometric morphometric analysis through computed microtomography. These methods and the geometric morphometric analyses of these mutants have all been published previously (Hallgrímsson et al. 2006; Hallgrímsson et al. 2007a, 2007b; Lieberman et al. 2008). Figure 5 summarizes these results for both mutants.

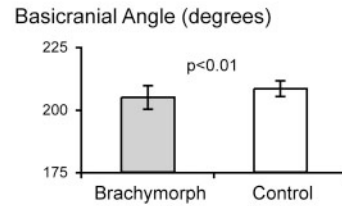
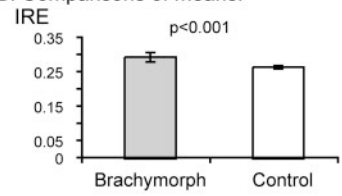
In both cases, the increase in brain size relative to cranial base length produces an increased flexion (or decreased retroflexion) of the cranial base, as predicted by the spatial packing model.

I. Brachymorph Mutant

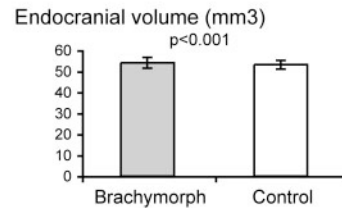
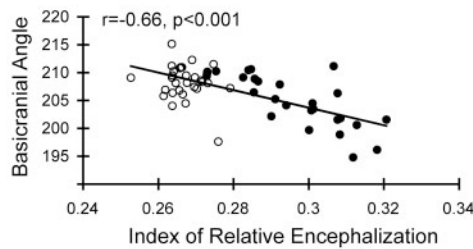
A. Mean Shapes



B. Comparisons of means.

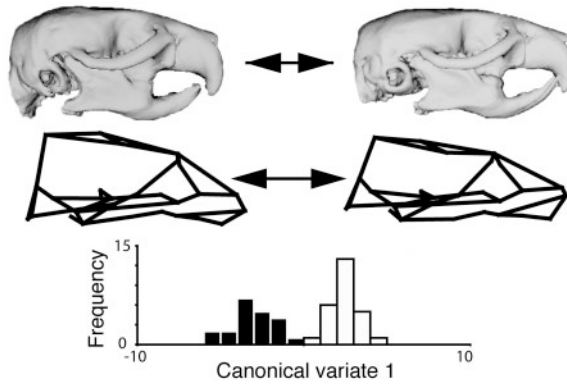


C. Cranial base angle against endocranial volume.

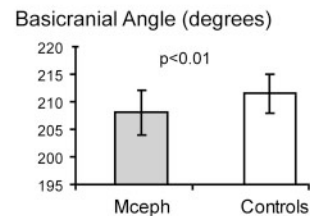
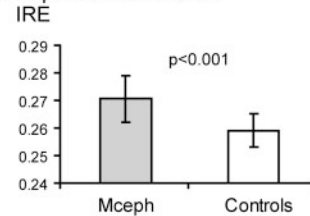


II. Mceph Mutant

A. Mean Shapes



B. Comparisons of means.



C. Cranial base angle against endocranial volume.

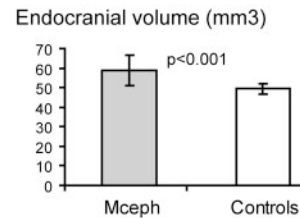
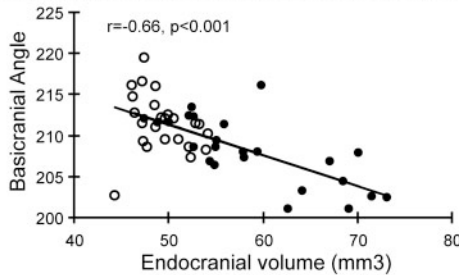


Fig. 5 Summaries of the analysis of the BM (A) and mceph (B) mutants. In both cases, (i) shows the generalized shape images showing the mean morphology for both genotypes as well as the results of canonical-variates analysis as well as the results of canonical variates analysis showing the shape differences between the groups. Principal components analysis revealed very similar results. The generalized shape images were obtained as described by Kristensen *et al.* (2008). This refers to the mean shape as obtained through scaling, superimposition, and averaging of the volumetric image data for the entire sample. (ii) shows means for key variables related to spatial packing that differ significantly between the genotypes. (iii) shows the relationship between angle of the cranial base and relative encephalization for the BM mutant and brain size for the mceph mutant. Throughout the figure, filled circles or bars indicate the mutant while open circles or bars indicate wild types (BM) or heterozygotes (mceph).

In both cases, these effects are also almost certainly epigenetic. The *Kcna1* mutation has no known effects on cartilage growth and is thus unlikely to exert any direct effect on the growth of basicranium. The change in cranial base angle is, therefore, a secondary and likely epigenetic effect of the increase in brain size. In the case of the BM mice, the *Papps2* gene has no effect on brain growth (Hallgrímsson et al. 2007a) and is not expressed in the brain (Alnouti and Klaassen 2006). Therefore, the greater flexion of the cranial base in these mutants is also likely an epigenetic consequence of the smaller cranial base relative to an unaltered brain.

A third mutant model relevant to the question of how the brain and cranial base are integrated is the Cre-Lox driven tissue-specific knockout of the *Pten* (tumor-suppressor phosphatase with tensin homology) gene. *Pten* negatively regulates the phosphatidylinositol 3' kinase signaling pathway that is responsible for controlling proliferation and size of chondrocytes as well as their differentiation and survival (Sansal and Sellers 2004). In *Pten*^{flox/flox} × *Col2a1*-Cre mice cartilage growth is increased, resulting in increased growth of endochondral bone. (Ford-Hutchinson et al. 2007). In the *Pten* skull, increased growth should occur only in the chondrocranium. Therefore, these mice should exhibit increased basicranial length relative to brain size and thus exhibit the opposite result compared to the other two mutants.

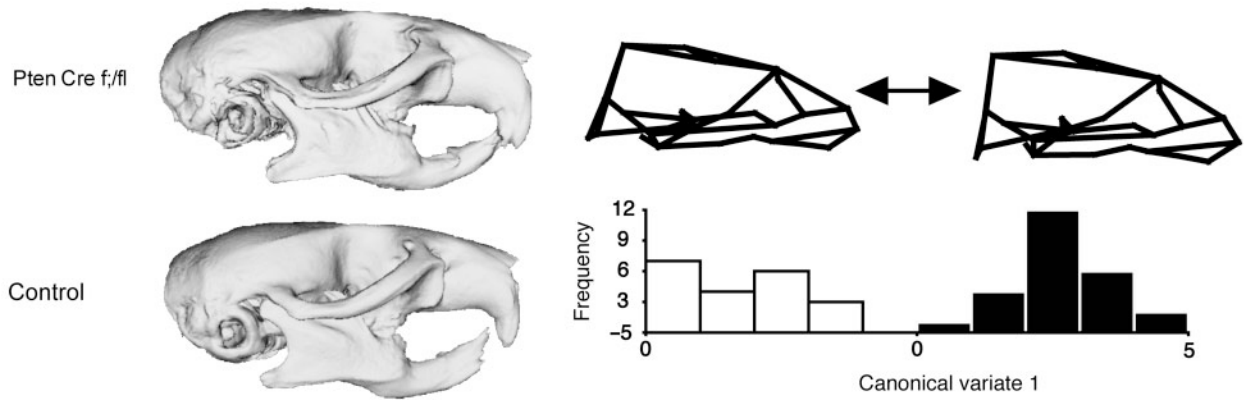
The results of the analysis of the *Pten* mutant revealed a more complex picture than we anticipated, but one that is nonetheless consistent with the spatial-packing model (Lieberman et al. 2008). It turns out that an unanticipated effect of the *Pten* mutation on growth plates and synchondroses is a disorganization of the more rapidly proliferating chondrocytes, along with premature mineralization, thereby leading to occasional premature fusion of the joint (Ford-Hutchinson et al. 2007). In our sample, therefore, basicranial synchondroses apparently fused prematurely. This fusion may account for the surprising result that while the anterior basicranium is elongated in the *Pten* mutant, the posterior basicranium is actually shorter (Lieberman et al. 2008) (Fig. 6). Since the premature fusion of growth plates in the *Pten* mutants affects only some joints within some individuals, the mutation also produces a significant (three-fold) increase in variance in shape (Fig. 6). While the average ratio of brain size and cranial-base size is not influenced by the mutation, this increased variance is reflected in a correlation between relative encephalization and the

angle of the cranial base within the genotype (Lieberman et al. 2008) (Fig. 6).

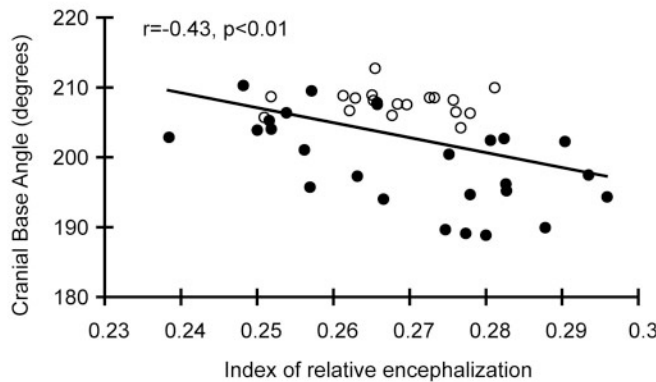
As one might expect, the spatial-packing story is actually even more complicated, leading us to expand the model in two ways (Lieberman et al. 2008). Using 3D data, we added width to what essentially has been a 2D model in previous studies. We have also taken into account the potential interaction between the angle of the cranial base and the size, particularly length, of the face in relation to the length of the anterior cranial base. Relatively longer faces may be correlated with more extended crania because of the need to accommodate the face and pharynx (Lieberman et al. 2000a, 2000b) or because of constraints on the orientation of the cribriform plate relative to the face (Enlow and Azuma 1975; Ravosa and Shea 1994; McCarthy 2001). Expanding the model to include these factors, we were able to explain the vast majority (87%) of the variation in angle of the cranial base among genotypes and strains of mice (Lieberman et al. 2008). This impact of variation in the length of the face relative to the anterior cranial base is also seen in the *Pten* mutant where relatively longer faces are associated with extension of the cranial base's angle (Fig. 6C). These results show unambiguously that spatial packing of the brain and, to a lesser degree, of the face, drives a large proportion of its variation from the angle of the cranial base.

What do these results mean for primate and, particularly, human evolution? The mutant mouse models described above have mutations that affect the growth of either the chondrocranium or the brain. It would be absurd to suggest that mutations related to sulfation, such as the *Pten* pathway, or voltage-gated channel proteins were involved in the evolutionary changes that led to human craniofacial form. But what these models do show is that changes in brain size relative to cranial-base size produce variation in a major aspect of human craniofacial form—the angle of the cranial base—via epigenetic interactions among key components of the developing head. The studies conducted thus far are not definitive. More work is needed to determine the developmental mechanisms by which the cranial base, via rates of cell proliferation at the synchondroses or modeling of the elements of the cranial base, responds to variation in brain size or in facial length. However, the findings thus far are very suggestive. They imply that evolutionary changes in the spatial-packing variables in mice and, presumably, in other mammals, would produce predictable variation in angle of the cranial base and this variation would be produced regardless of the

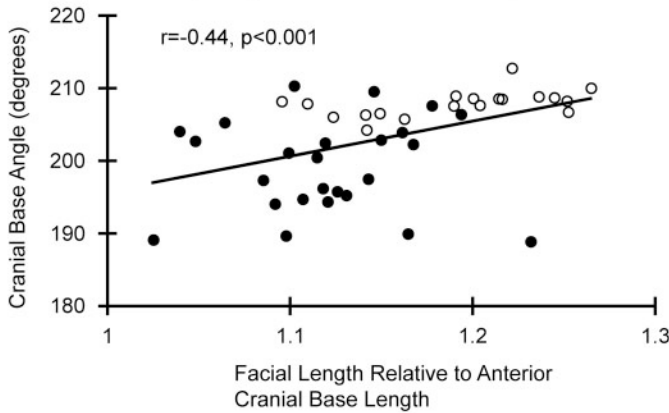
A Mean Shapes



B Cranial base angle against IRE



C Cranial base angle against relative facial length



D Comparisons of means.

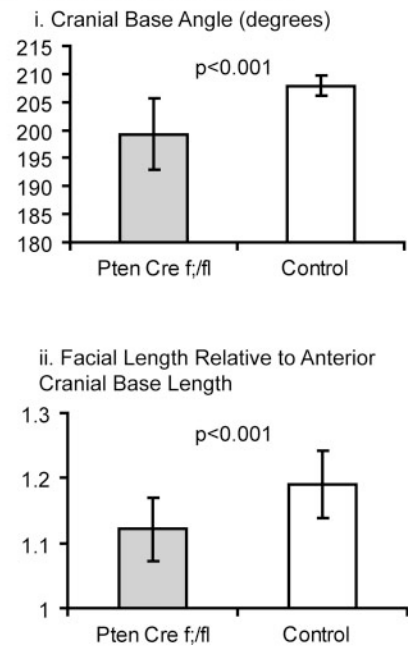


Fig. 6 Summary of the morphological analysis of mice with a tissue-specific knockout of the *Pten* gene. (A) Generalized shape images showing the mean morphology for both genotypes as well as the results of canonical variates analysis showing the shape differences between the groups. Principal components analysis revealed very similar results. (B) The relationship between angle of the cranial base and relative encephalization (IRE), which is calculated as the cube root of endocranial volume divided by basicranial length. (C) The relationship between the cranial base's angle and facial length relative to anterior length of the cranial base. (D) The means for angle of the cranial base and relative facial length for both genotypes.

specific mutations that generated those changes. If true, then this pattern of epigenetic interactions means that the unusually flexed basicranium of the human skull, which produces our unusually orthograde face, does not require a

special adaptive explanation. It arises, rather, as a by-product, to some degree, of our smaller faces, but mostly because of the need to accommodate our enlarged brain on a relatively small cranial base.

Conclusion

Using model organisms such as the mouse to address questions in evolutionary developmental biology is complicated in two fundamental ways. The first is that the phenomena which relate development to evolution, such as integration, modularity, constraints, and canalization, are complex. Consequently they are not only difficult to study in model organisms, such as mice, but are even more difficult to extrapolate to other organisms, such as primates or humans. Applying what we've learned about these phenomena to the explanation of evolutionary change in specific lineages is more difficult still. A fundamental insight into the developmental determinants of canalization in inbred mice, for instance, may lead to a general theoretical appreciation for the potential evolutionary significance of canalization but not necessarily to an understanding of the role that canalization has played in the evolution of humans or walrus. Secondly, evolutionary change is by definition historically contingent and, at some level, unrepeatably (Williams 1966; Gould 1989). If one could repeat the evolution of some lineage under the same selection gradients, one would almost certainly end up with different mutations affecting different pathways each time. Our point here is that a solution to both of these problems is to compare model organisms to evolutionary lineages of interest at the appropriate level in the developmental hierarchy for the questions being asked. To understand how changes in size of the brain may have influenced overall craniofacial form, it is still appropriate to use mutant models with increased brain size or reduced cranial-base size, even though the developmental-genetic basis for the evolutionary change is radically different. Mutations of major effect, like those analyzed here, are unlikely to contribute much to evolutionary change. However, the developmental processes through which they exert their phenotypic effects are the same processes influenced by evolutionarily significant genetic variation. As by the example of the brain and cranial base, these process-to-phenotype relationships are no less ontologically relevant than are genetic factors for understanding the developmental basis for evolutionary change and the role of development in affecting evolutionary change. Pursuing this level of explanation offers a means to apply an experimental approach to the evolutionary developmental biology of primates and thus opens the door to unique insights

that will enhance our understanding of our evolutionary past.

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