Developmental regulation of skull morphology. I. Ontogenetic dynamics of variance

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SUMMARY In the absence of processes regulating morphogenesis and growth, phenotypic variance of a population experiencing no selective mortality should increase throughout ontogeny. To determine whether it does, we measure variance of skull shape using geometric morphometrics and examine its ontogenetic dynamics in the precocial cotton rat (*Sigmodon fulviventer*) and the altricial house mouse (*Mus musculus domesticus*). In both species, variance of shape halves between the two youngest samples measured (between 1 and 10 days postnatal and 10 and 15 days postnatal, respectively) and thereafter is nearly constant. The reduction in variance did not appear to result from a general regulation of skull size or developmental timing, although skull size may also be regulated and developmental timing is an important component of the variation in skull shape of young house mice. The ontogenetic dynamics of variance suggest two possible scenarios. First, variation generated during fetal or early postnatal growth is not immediately compensated and therefore accumulates, whereas later in growth, variation is continually generated and rapidly compensated. Second, variation generated during fetal and early postnatal growth is rapidly compensated, after which no new variance is produced. Based on a general model for bone growth, we hypothesize that variance is generated when bone grows under the direction of disorganized muscular movements and decreases with increasing neuromuscular control. Additionally, increasing coherence of signals transmitted by the growing brain and sensory organs, which exert tensile forces on bone, may also canalize skull shape.

INTRODUCTION

Canalization refers to the buffering of developmental systems so that the same phenotype is produced despite genetic and environmental variation in the population (Waddington 1942). In many cases, canalization might prevent variation from being generated in the first place and thus represents a generative constraint as defined by Wagner and Misof (1993). But canalization need not prevent variation from being generated in the first place; instead, it could restore deviants to the mean, as in the case of targeted growth. When growth is targeted, individuals who are small for their age grow atypically fast or for longer than average, thereby attaining normal adult body size (Tanner 1963; Monteiro and Falconer 1966; Riska et al. 1984; Shalitin and Phillip 2003). Targeted growth is so typical of mammals that it can be considered a basic characteristic of their ontogeny, and it is so representative of a canalized process that Waddington (1952) exemplified canalization of quantitative traits by adult body size.

Processes like targeted growth reduce variance over ontogeny, and that reduction indicates developmental regulation because, in the absence of regulatory processes, we would expect the opposite trend—an ontogenetic increase in variance. That increase is found for deviations from bilateral asymmetry of skeletal form over postnatal ontogeny (as measured by fluctuating asymmetry; Hallgrímsson 1998, 1999), suggesting that these are not compensated. In contrast, variance in skull size decreases over ontogeny of laboratory rats (Nonaka and Nokata 1984), and variance of skull shape also reportedly decreases in the only two species examined, cotton rats (Zelditch et al. 1993) and another sigmodontine rodent, *Calomys expulsus* (Hingst-Zaher et al. 2000).

That skull shape would be canalized is not surprising considering that the cranium houses the brain and vital sensory organs and that skull bones function as struts and levers that must be properly placed to function effectively, if only to align occluding teeth. However, skull shape is molded by complex interactions that might be differently coordinated across individuals. These interactions ensure that the calvarium is just large enough to enclose the brain, for example, and that bones are strong enough to resist being deformed when loaded by muscles. Such interactions regulate the form of

developing parts within individuals but do not need to reduce variance among individuals. They may even increase it, as they apparently can increase fluctuating asymmetry (Hallgrímsson 1998, 1999).

To explain how skull shape could decrease in variance even as fluctuating asymmetry increases, Hallgrímsson (1999) hypothesized that the variance in skull shape might result from variation in developmental timing, that is, from variation in the position of individuals along the normal ontogenetic trajectory. That hypothesis is supported by the observation of high variance of developmental timing in mammalian fetuses (Hall and Miyake 1995; Miyake et al. 1996), and it is plausible because a reduction in this component of variation would not affect random deviations from bilateral symmetry. Similarly, a reduction in the variance of skull size might also reduce variance in skull shape without affecting random deviations from bilateral symmetry.

We might expect developmental timing and size to be highly regulated, even more so than shape, because developmental timing and size are likely to be highly consequential to Darwinian fitness; minor departures from the norm might have a greater impact on fitness than minor deviations in skull shape. In particular, neonatal maturity of precocial mammals is likely to be under intense stabilizing selection because the maturity of neonates is the defining trait of precociality. Of the two species that reportedly reduce their variance in skull shape over ontogeny, one is the precocial cotton rat *Sigmodon fulviventer* (Zelditch et al. 1993). However, the reported reduction of variance in that species is questionable because variance was not rigorously quantified; the inference of reduced variance was based on graphically depicted ellipses of variation for individual variables (that are not individually meaningful). The other species for which a reduction of variance is reported is the altricial *Calomys expulsus* (Hingst-Zaher et al. 2000), and this reduction is also questionable although on different grounds. In that case, the dramatic reduction coincides with a major transition in methods of specimen preparation: from measuring cleared and stained skulls to measuring skeletonized skulls. By itself, a reduction in preservational artifacts could reduce variance.

Our primary objective herein is to examine the ontogenetic dynamics of variance in skull shape to test the hypothesis that it does decrease over ontogeny. Our secondary objective is to test the hypothesis that shape is canalized indirectly, either by reducing the variance in developmental timing or size. These two factors are not necessarily equivalent because individuals who are small for their age are not necessarily immature, so we consider them separately and also measure their joint contribution to the variance in skull shape. We compare two species, cotton rats, the subject of a previous study (Zelditch et al. 1993), and house mice *Mus musculus domesticus*, the favored model for developmental and genetic studies of mammalian skulls. Unlike cotton rats, house mice are altricial and are thus more representative of rodents (other than hystricomorphs) and are also less likely to be under stabilizing selection for an optimal degree of neonatal maturity.

MATERIALS AND METHODS

Samples

Our sample of cotton rats (*S. fulviventer*) comprised offspring of wild-caught parents, bred and reared in the Michigan State University Museum, and killed at 10-day intervals, starting the day of birth. Table 1 gives sample sizes and numbers of litters from which individuals were sampled for each age. These are the same individuals as analyzed in previous studies (Zelditch et al. 1992, 1993) except for the addition of two older cohorts (40 and 50 day olds). Age classes were sampled haphazardly rather than randomly; it is not possible to determine from colony records the scheme whereby individuals were selected for sacrifice at a particular age. For the two oldest cohorts, complete pedigree information is lacking, so in counting the numbers of litters we assume that individuals born on different days come from different litters and that those born the same day come from the same litter. Thus, for the two oldest cohorts, the number of litters from which individuals were sampled could be an underestimate if two or more litters were born the same day.

No natural deaths were recorded for any young of this species except for the occasional individual found outside the cage or killed by relatives (none of which is included in the sample). We measured every other known-age individual available in the collection except for those with badly damaged skulls. These samples are heterogeneous with respect to geographic origin of the parents and sex (although sexual dimorphism is subtle in this species and was not detected in these samples) and include litter mates within cohorts. Because of the geographic heterogeneity of the sample, analyses were done separately for geographically homogeneous subsamples; because the results do not differ from those based on the pooled sample and sample sizes are too small for statistical tests, they are not separately reported.

Our parental stock of house mice (*M. m. domesticus*) is the Hsd/ICR strain, obtained from Harlan Sprague Dawley (Harlan, Indianapolis, IN, USA). This out-bred laboratory stock has been used in a previous study of compensatory growth (Riska et al.

Table 1. Sample sizes and numbers of litters included within each sample for each age for each species

Age	*Sigmodon fulviventer*	*Mus musculus domesticus*
1	18/9	—
10	18/10	25/21
15	—	21/21
20	17/14	15/15
25	—	15/15
30	18/12	13/13
40	11/7	25/20
50	12/8	29/22

1984) as well as in studies of the evolution of ontogenies of voluntary activity and its consequences for body weight and food consumption (Morgan et al. 2003). Mice were bred, reared, and killed at the University of Wisconsin under the supervision of T. Garland; skeletons were prepared at the Museum of Zoology, University of Michigan. Because the skulls of neonatal mice are poorly ossified, we could not measure them; thus the youngest mice analyzed herein are 10 day olds. Samples were taken at 5-day intervals thereafter through 30 days and then at 10-day intervals until 50 days. Table 1 gives sample sizes and numbers of litters from which individuals were sampled for each age. These samples are also heterogeneous with respect to sex and include litter mates within cohorts.

Because these species differ in life history, age-based comparisons are problematic whether they are based on gestational or postnatal age. The species differ in gestation length (31 vs. 19 days for cotton rats and house mice, respectively) and also in developmental rate, which is significantly lower in cotton rats than in house mice (Zelditch et al. 2003). Thus, house mice are far less mature at birth but gain ground postnatally, such that the two species undergo weaning and reach sexual maturity at the same postnatal ages. To put age-based comparisons in developmental context, we can use a framework based on degree of skull shape maturity relative to asymptotic adult maturity (Table 2) calculated from a model for maturation of shape (Zelditch et al. 2003). Based on the parameters of the model, 1-day-old cotton rats are comparable with 10-day-old house mice in degree of maturity and are visibly similar in degree of skull ossification (Fig. 1); 10-day-old cotton rats are approximately comparable with 15-day-old house mice, and 20-day-old cotton rats are approximately comparable with 20-day-old house mice. From that point on, the two species are nearly equal in degree of maturity at any given age.

Table 2. Degree of maturity of skull shape, given as a proportion of the asymptotic (adult) maturity, at sampled postnatal ages, according to predictions of the best-fitting model for developmental rate

Age	*Sigmodon fulviventer*	*Mus musculus domesticus*
1	0.36	− 0.17
5	0.46	0.12
10	0.57	0.38
15	0.65	0.56
20	0.72	0.69
25	0.77	0.78
30	0.82	0.85
35	0.85	0.89
40	0.88	0.92
45	0.90	0.95
50	0.92	0.96

From Zelditch, M. L., Lundrigan, B. L., and Sheets, H. D. 2003. Do precocial mammals have a faster developmental rate? A comparison between *Sigmodon fulviventer* and *Mus musculus domesticus*. *J. Evol. Biol.* 16: 708–720.

Morphometric Methods

To examine the variance in skull shape and size, we use landmark-based, geometric, morphometric methods. Landmarks were sampled on skulls skeletonized by dermestid beetles and photographed in palatal view, with the occlusal surface of the molars oriented parallel to the photographic plane (Fig. 2, A and B). Specimens were supported by modeling clay and orientations were initially checked by eye then rechecked with a ruler; a level was used to orient the camera to the tabletop. Selected landmarks differ between species because some could not be reliably located in both, although there is a large subset of landmarks common to both species that can be used in comparative analyses (i.e., all those shown on the skull of house mice [Fig. 2B] with the exception of the interior corner formed by the intersection of the zygomatic arch with the braincase [ZA]). Although the subset of landmarks common to both provides a basis for comparison, it does not fully capture the shape of the skull, so analyses were done using the complete set of landmarks for each species and the subset common to both.

All landmarks were digitized on both sides of the skull, and bilaterally homologous landmarks were then averaged to avoid inflating degrees of freedom. This procedure precludes analyzing fluctuating asymmetry but allows us to include individuals that are damaged on one side. To ease interpretation of the graphic results, all are shown for the whole skull by reflecting the averaged landmarks back over the midline.

Shape analyses were done by superimposing configurations of landmarks using the Generalized Least Squares Procrustes superimposition (GLS), which preserves all information about shape differences among specimens, removing only the information unrelated to shape (i.e., scale, position, and orientation; Rohlf and Slice 1990). This superimposition minimizes the Euclidean distance between shapes, which is calculated as the square root of the squared distances between homologous landmarks summed over all landmarks. The minimum distance that can be obtained by translation, scaling, and (rigid) rotation of specimens is the

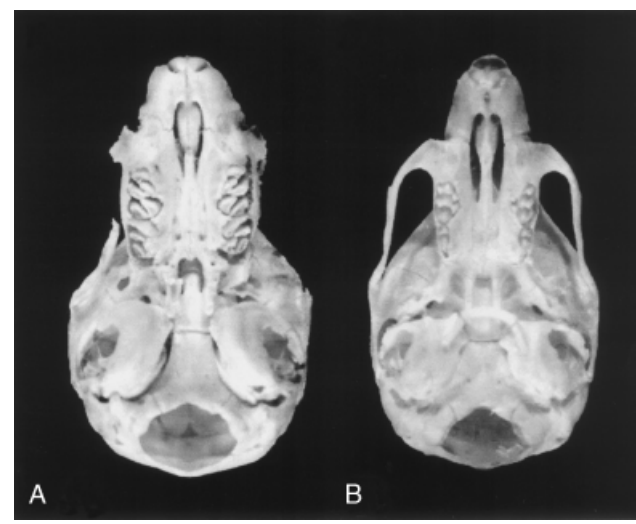
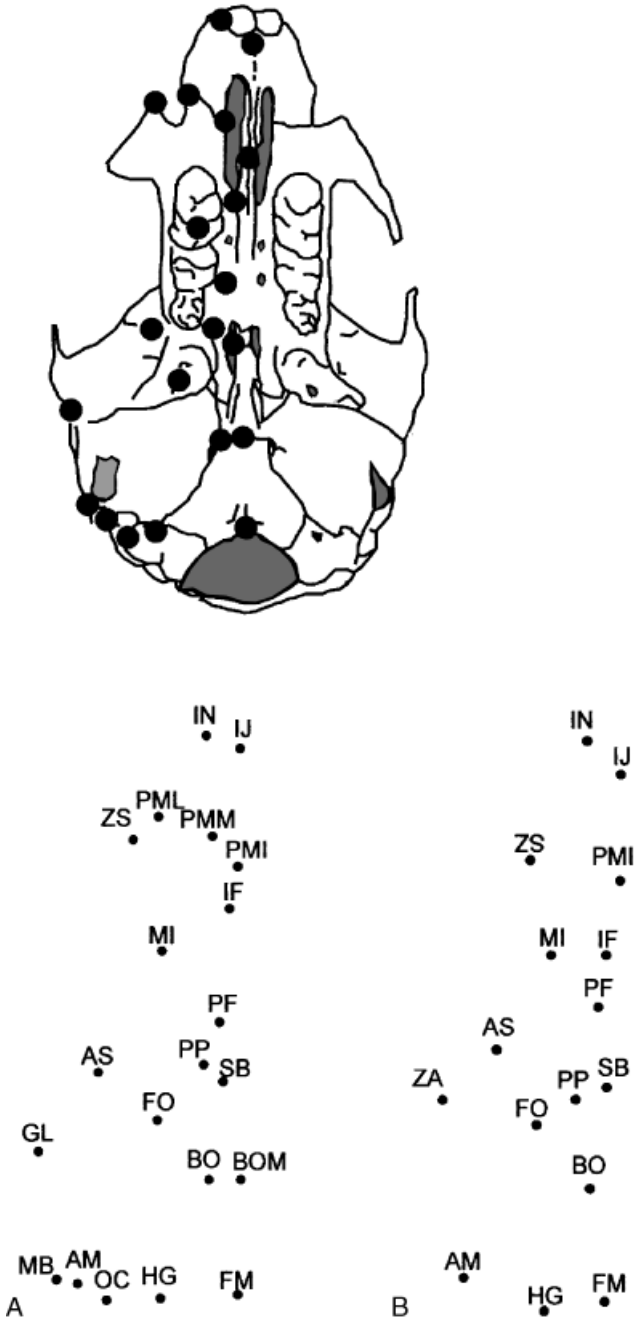


Fig. 1. Skulls of (A) 1-day-old *Sigmodon fulviventer* and (B) 10-day-old *Mus musculus domesticus*.

Procrustes distance, the conventional metric for overall shape dissimilarity in geometric morphometrics (Dryden and Mardia 1998). Analyses of skull size used the standard measure of geometric scale, centroid size (CS), defined as the square root of the squared distance between each landmark and the centroid of the landmark configurations summed over all landmarks. GLS superimposition and the calculation of CS were done in CoordGen, part of the Integrated Morphometrics Programs (IMP), produced in Matlab6 (Mathworks 2000). Compiled stand-alone versions running in Windows are freely available at http://www2.canisius.edu/~sheets/morphsoft.html.



Estimating variance of shape and size

To estimate the variance of shape, we use the standard formula for a variance:

$$V = \frac{\sum_{j=1}^{j=n} d_j^2}{(n-1)} \quad (1)$$

where d_j is the Procrustes distance of individual j from the mean shape for its age and n is the sample size for an age class. Because the distance metric is Euclidean, V is also the trace of the variance–covariance matrix of shape variables (i.e., the sum of their univariate variances). In these calculations, it does not matter whether the variances are computed from the shape coordinates obtained by the Procrustes superimposition or from other geometric shape variables such as the partial warp scores (including the scores on the uniform component) because they yield the same estimates of the distance.

Analyses of the ontogenetic dynamics of shape variance were done using two approaches. The first compares the variances between successive age classes using a t-test (standard errors of shape variance were estimated by bootstrapping). The second examines evidence for ontogenetic trends in variance by regressing variance on age; this allows for detecting ontogenetic changes that fail to reach statistical significance in comparisons between successive ages. To exclude the possibility that an apparent trend is due to the extreme values in the youngest age classes, that sample was removed from the analysis. Calculation of shape variance and its standard error was done by DisparityBox, another program in the IMP series.

Fig. 2. Landmarks sampled on skulls of both species: (A) *Sigmodon fulviventer* and (B) *Mus musculus domesticus*. Descriptions of landmarks and abbreviations are as follows: for *S. fulviventer*: juncture between incisors on premaxillary bone (IJ); premaxilla-maxilla suture where it intersects outline of the skull in photographic plane (PML); lateral margin of incisive alveolus where it intersects outline of the skull in photographic plane (IN); anteriormost point on the zygomatic spine (ZS); suture between premaxillary and maxillary portions of palatine process (PMI); premaxilla-maxilla suture lateral to incisive foramen (PMM); posteriormost point of incisive foramen (IF); median mure of first molar (MI); posterior palatine foramen (PF); posterolateral palatine pit (PP); junction between squamosal, alisphenoid and frontal on squamosal-alisphenoid side of suture (AS); midpoint along posterior margin of glenoid fossa (GL); anteriormost point of foramen ovale (FO); lateralmost point on presphenoid-basisphenoid suture where it intersects the sphenopalatine vacuity in the photographic plane (SB); the most lateral point on basisphenoid-basioccipital suture (BO); midpoint of basisphenoid-basioccipital suture (BOM); hypoglossal foramen (HG); juncture between paroccipital process and mastoid portion of temporal (OC); midpoint of foramen magnum (FM); juncture of mastoid, squamosal, and bullae (MB); juncture between mastoid and medial end of auditory tube (AM). For *M. m. domesticus*: a subset of the landmarks described above, with the interior corner formed by intersection of zygomatic arch with braincase (ZA). The set of landmarks common to both species include all those visible on *M. m. domesticus* with the exception of ZA.

To estimate the variance of size, we calculated the variance of CS, ln-transforming it to remove the correlation between mean and variance. Comparisons between variances of successive ages were done using Levene's test for the equality of variances (Levene 1960). We use this rather than the F-test because it is less sensitive to departures from normality (Van Valen 1978). The test is performed by calculating the absolute value of the deviation of each individual from the sample mean; the means of the deviations are compared by a *t*-test. To determine whether variance shows a trend related to age, size variance was regressed on age. Both Levene's test and regressions were done in Microsoft Excel.

Estimating the variance explained by size and developmental timing

To determine whether the variance in shape is regulated indirectly via the regulation of size and developmental maturity, we estimated the proportion of shape variance within each age class explained by size and maturity. These are not mutually exclusive hypotheses because an individual can be both unusually large and unusually mature for its age, but neither are they perfectly correlated, because an individual can be both atypically small and atypically mature for its age. Thus, we first estimate the proportion of shape variance explained by size and then by developmental maturity, and we then remove the variance explained by size and recalculate the proportion of the residual explained by maturity.

Estimating the proportion of shape variance explained by size is straightforward because we have a simple (scalar) measure of size that can be used in a multivariate regression—CS. This regression is done by treating the complete set of shape variables as the dependent variable, which is regressed on size. More specifically, the dependent variable is the full set of partial warp scores, including scores on the uniform component, a convenient set of shape variables for multivariate analyses because the number of variables equals the degrees of freedom for the statistical analysis. To evaluate the fit to the regression model, we use a generalization of Goodall's (1991) *F*-ratio:

$$F = \frac{\sum d^2_{x\bar{x}}/q}{\sum d^2_{x\bar{x}}/(n-q-1)} \qquad (2)$$

where $d^2_{x\bar{x}}$ is the squared deviation between the shape of a specimen, x, and the shape expected for its size, $\bar{x}$. The magnitude of that deviation is measured by the Procrustes distance between the two shapes. The parameter q is the number of independent variables, and n is sample size. This ratio is analogous to the ratio of the explained to unexplained variance in a regression (Rohlf 1998). The statistic is compared with an *F*-distribution with qm and $(n-q-1)m$ degrees of freedom, where n and q are as defined above and m is the dimensionality of the shape space, which equals twice the number of landmarks minus four. Goodall's *F*-test was done using TpsRegr (Rohlf 1998), freely available at http:/life.bio.sunysb.edu/morph.

No such straightforward method can be used to quantify the shape variance explained by developmental maturity because there is no simple (scalar) measure of developmental maturity that can serve as the independent variable in a regression. No single shape variable provides a proxy for degree of maturity because individuals might differ from the mean in any shape variable due to variation in the ontogeny of shape and to variation in position along the mean trajectory. Therefore, to quantify the variation explained by the trajectory, we calculate the proportion of variance lying along it, a procedure that involves constructing the ontogenetic trajectory of shape (by regressing shape on age) using a piece-wise linear regression. Piece-wise regression is used because the ontogenetic trajectory is not linear in shape space—it curves (Zelditch et al. 2003). For that reason, we calculate two trajectories for each sample, the one extending from the next younger age to the focal sample and the other extending from the focal sample to the next older age, and estimate the proportion of shape variance explained by each. To calculate that proportion, we calculate the correlation between an ontogenetic vector and each principal component (PC) of within-age variance; that correlation is estimated by the dot (inner) product between a PC and a trajectory (both vectors normalized to unit length). The square of the vector correlation gives the proportion of the variance of a single PC explained by its correlation with the ontogenetic trajectory, which must be weighted by the eigenvalue of the component to estimate the variance due to developmental timing. Summing that quantity over all components and dividing by the total variance gives the proportion of the total explained by variation in developmental timing along one trajectory; as our estimate of the variance explained by developmental timing we used the trajectory yielding the larger value. To check the accuracy of these estimates, we used this approach to estimate the proportion of variance explained by size and compared those estimates with the ones obtained by the more straightforward regression method explained above. In these analyses, we calculated correlations between PCs and the vectors of within-sample allometric coefficients (i.e., the coefficients of static allometry). The results of the two methods are nearly identical; for example, for the sample of 10-day-old house mice, the two methods yield 8.95% versus 8.96%.

Because size and developmental maturity could be explaining some of the same variance, we calculated the proportion of variance explained by developmental timing twice, once without removing the size-related variance and once after removing it. To remove the variance explained by size, we regressed shape on size (for each age class separately), which gives an estimate of the expected shape for a given size; in these analyses, we use the mean size of each sample to predict the expected shape. The residuals from the regression are added to the expected shape. To determine the total variance explained by size and/or developmental timing, we summed the proportions of variance explained by size and by size-independent developmental timing. Regressions were done using Regress6, PCs analysis by PCAGen, and size standardization by Standard6, all programs in the IMP series. The calculation of vector correlations and of the proportion of shape variance explained by the ontogenetic trajectory were done in Microsoft Excel (Microsoft Corp., Redman, WA, USA).

RESULTS

The ontogenetic dynamics of shape variance

Variation in landmark locations appears to be unequally distributed across both ages and landmarks in cotton rats

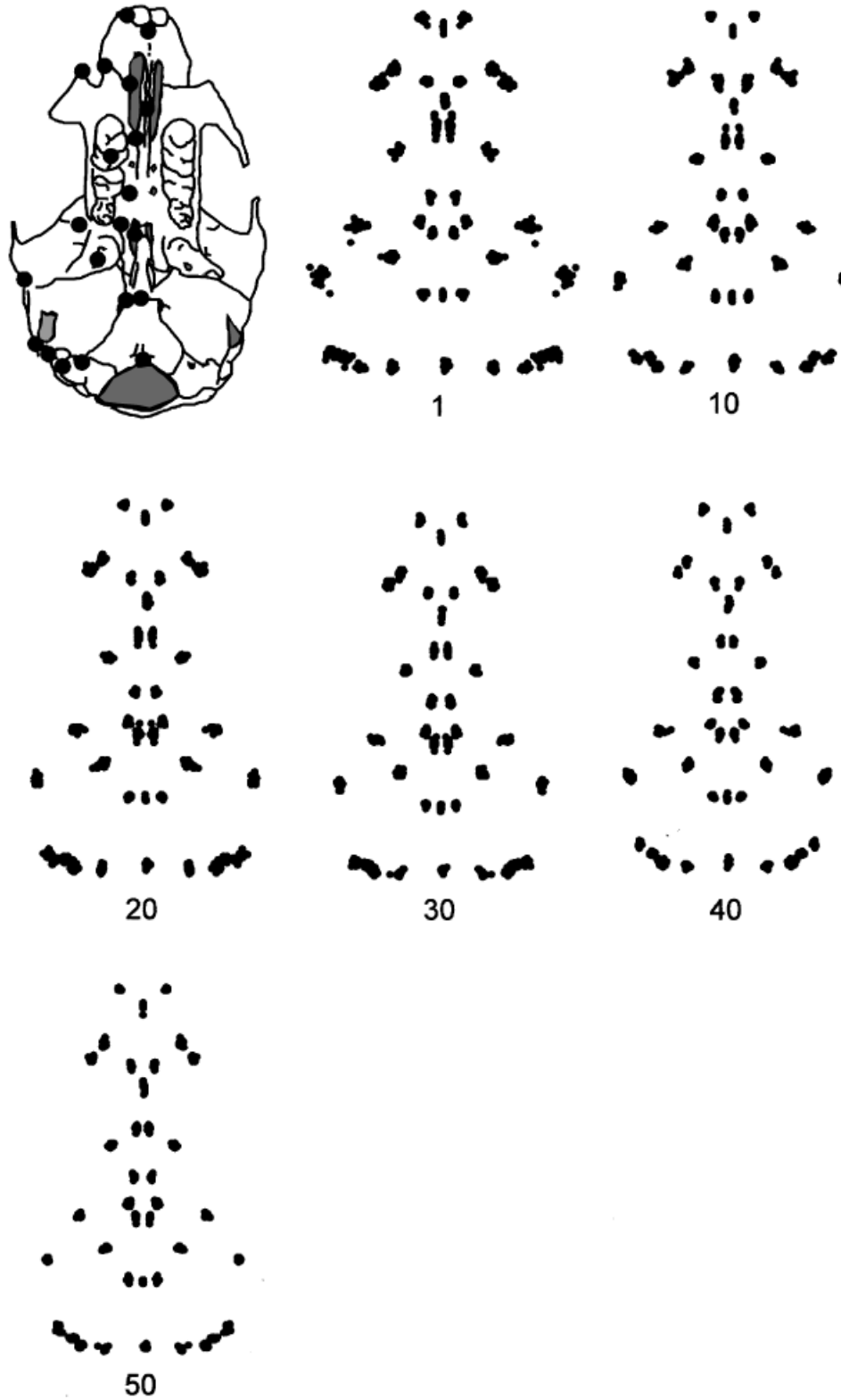


Fig. 3. Variability of skull shape in each age class of *Sigmodon fulviventer*. The data for the complete set of landmarks are shown, without removing effects of size. Landmarks are superimposed by a Procrustes Generalized Least Squares superimposition (ages are given below the configurations).

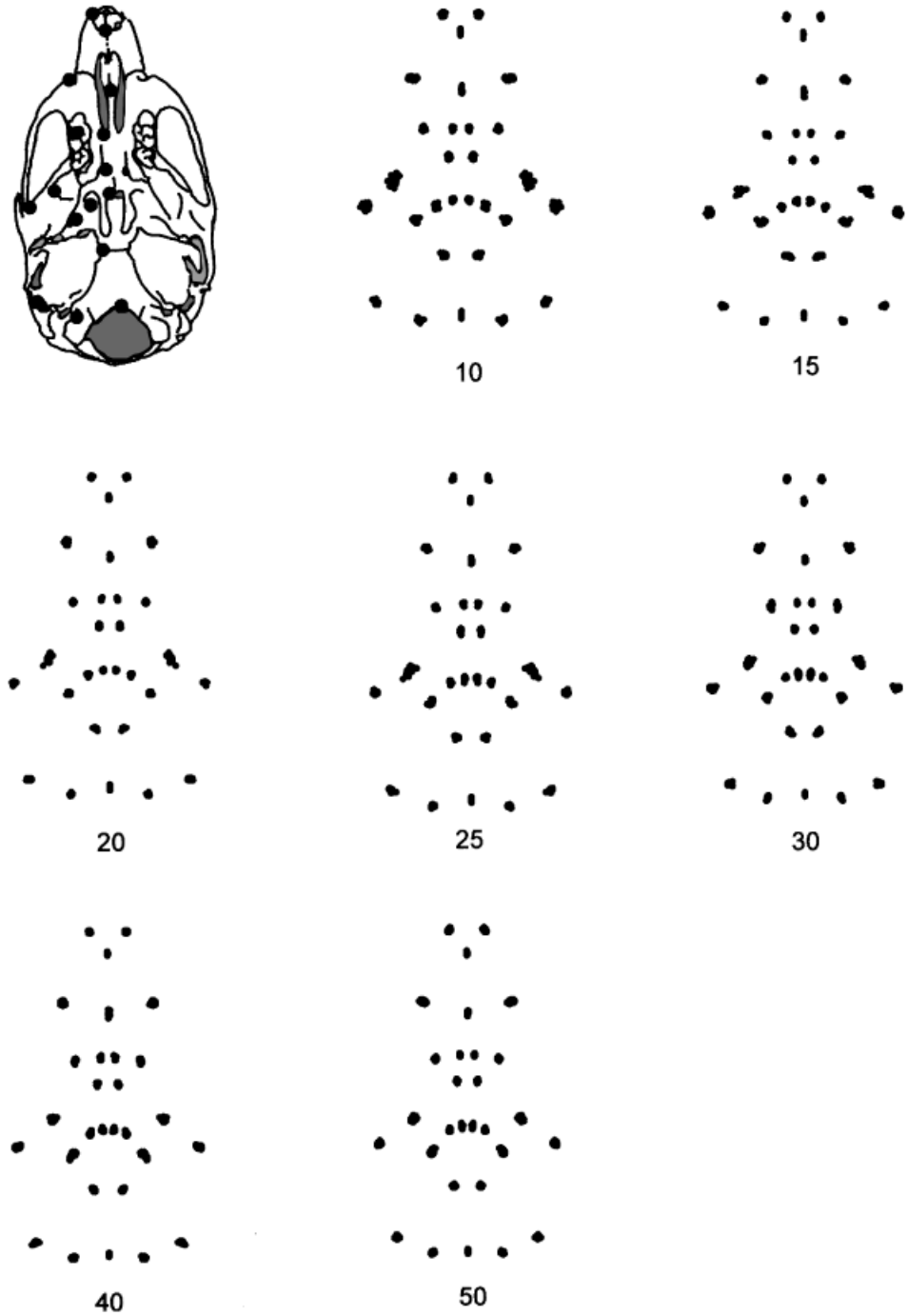


Fig. 4. Variability of skull shape in each age class of *Mus musculus domesticus*. The data for the complete set of landmarks are shown, without removing effects of size. Landmarks are superimposed by a Procrustes Generalized Least Squares superimposition (ages are given below the configurations).

(Fig. 3) and house mice (Fig. 4), a visual impression confirmed by statistical analysis. In both species, variance is approximately halved between the youngest and next youngest age class examined (Table 3), a statistically significant decrease (see t values, Table 4). Thereafter, no two successive age classes differ significantly from each other, with the exception of the decrease from 30 to 40 days in house mice. However, there does appear to be a slight trend toward decreasing

Table 3. Variance in skull shape at each age (measured from birth) for the complete data set (comprising all landmarks) and for the subset of landmarks common to both species

	Sigmodon fulviventer		*Mus musculus domesticus*	
Age	Complete	Common Subset	Complete	Common Subset
1	1.318	1.440	—	—
10	0.719	0.704	0.628	0.566
15	—	—	0.349	0.316
20	0.714	0.710	0.316	0.299
25	—	—	0.410	0.400
30	0.616	0.637	0.339	0.325
40	0.621	0.667	0.253	0.237
50	0.633	0.530	0.265	0.245

All variances are multiplied by 1000.

variance in both species, albeit not monotonic in either. The fluctuations around the trend are generally slight, with the exception of the unexpectedly high variance of the 25-day-old house mice. Even though the trend is not monotonic, variance is further reduced after the large drop between the two youngest ages; the second-youngest age is significantly more variable than the oldest ($P<0.05$ for both species and both data sets).

Although the two data sets yield the same general pattern and support the same statistical conclusions, the complete set of landmarks is usually more variable than the subset of landmarks common to both species. That is not surprising considering that the midpoint along the posterior margin of the glenoid fossa, GL (cotton rats) and the interior corner formed by the intersection of the zygomatic arch with the braincase, ZA (house mice) are highly variable (Figs. 3 and 4) and these are omitted from the common subset. When neither GL nor ZA contribute disproportionately to variance, the variance of the subset of landmarks common to both species is either higher or equal to that of the complete set. Aside from these landmarks specific to one species, others are consistently among the most highly variable in both species at all ages. The most notable of these is the junction between squamosal, alisphenoid, and frontal on the squamosal–alisphenoid side of suture (AS) and another is the anterior-most point on the zygomatic spine (ZS), although variance is not *at* these (or other landmarks); rather, it is the location of these landmarks relative to the others that varies. Beyond the similarity between species in the variability of AS and ZS, the species differ in the spatial distribution of variance. In cotton rats, two facial landmarks are exceptionally variable, albeit at different stages: the posteriormost point of incisive foramen (IF) through 30 days of age and the suture between premaxillary and maxillary portions of palatine process (PMI) from 30 days on (Fig. 3). None of the cranial or posterior basicranial landmarks is both exceptionally and consistently variable in this species, although some are highly variable in the youngest sample (e.g., juncture between mastoid and medial end of auditory tube [AM]). In contrast, the cranial landmarks are especially variable in house mice, particularly the most lateral point on the basisphenoid-basioccipital suture (BO) and the anteriormost point of foramen ovale (FO) (Fig. 4). Of the facial landmarks, only PMI and ZS are as variable as cranial landmarks (Fig. 4).

Table 4. *t* values for the comparisons between shape variances of successive age classes; standard errors of the estimated variances are obtained by bootstrapping

	Sigmodon fulviventer			*Mus musculus domesticus*	
Ages	Complete	Common	Ages	Complete	Common
1–10	4.57	4.58	10–15	3.82	3.43
10–20	0.05	0.05	15–20	0.53	0.28
20–30	1.11	0.68	20–25	1.24	1.34
30–40	0.06	0.32	25–30	1.11	1.33
40–50	0.14	1.69	30–40	2.50	2.34
			40–50	0.74	0.314

The two species differ not only in where variance is greatest, they also differ in the overall level of variance. Cotton rats are consistently more variable whether age is measured on a postnatal age scale (as in Table 3) or on a scale that takes gestational age and developmental rate into account, which would contrast 1-day-old cotton rats with 10-day-old house mice and 10-day-old cotton rats with 15-day-old house mice, after which the species are comparable at the same postnatal ages. For example, the variance of 1-day-old cotton rats is significantly higher than that of 10-day-old mice ($t = 6.30$; $P<0.0001$) as is also the case for the developmentally comparable 50 day olds ($t = 5.96$; $P<0.0001$).

The ontogenetic dynamics of size variance and its impact on variance in shape

Variation in size accounts for very little of the variation in shape, and variance in these two aspects of form follow different temporal patterns, although size, like shape, is far more variable in the youngest age class than in the next youngest (Table 5). That decrease, however, is statistically significant only for cotton rats (see *t* values in Table 6), and in that species, there is a second statistically significant decrease over the interval from 30 to 40 days. Not surprisingly, the null hypothesis of no linear relationship between age and size

Table 5. Variance of ln-transformed centroid size and the percent of shape variance within an age-class explained by size

	Sigmodon fulviventer		*Mus musculus domesticus*	
Age	Size Variance	Shape Explained (%)	Size Variance	Shape Explained (%)
1	2.74	9.66[1]	—	—
10	1.08	5.74	3.37	8.96[1]
15	—	—	1.87	8.32[1]
20	2.26	6.19	1.75	7.22
25	—	—	2.04	6.17
30	1.99	9.54[1]	1.96	8.70
40	0.47	11.43	1.48	3.78
50	2.31	15.57[1]	1.53	7.78[1]

Analyses are based on the subset of landmarks common to both species. All values for size variance are multiplied by 1000.
[1]Samples in which shape and size are statistically significantly correlated (at $P<0.05$).

Table 7. The proportion of shape variance explained by variation in developmental timing

	Sigmodon fulviventer		*Mus musculus domesticus*	
Age	With	Without	With	Without
1	12.43	10.85	—	—
10	10.08	10.70	27.83	29.61
15	—	—	10.35	10.37
20	5.43	2.89	32.42	23.94
25	—	—	33.96	27.84
30	7.75	5.25	16.80	12.05
40	6.93	7.42	9.22	7.76
50	6.98	8.16	11.76	10.07

Estimates are based on the subset of landmarks common to both species and are made including ("with") and excluding ("without") the variance explained by size.

variance cannot be rejected ($P = 0.63$). Nor is there a relationship between the magnitudes of variance in size and shape ($P > 0.30$ whether the youngest age class is included or not). In house mice, there is some evidence of a trend toward decreasing size variance over time, but as in cotton rats, the relationship between age and size variance is not statistically significant ($P = 0.08$). There is, however, a statistically significant relationship in house mice between levels of variance in size and shape ($P < 0.05$, whether the youngest age is included or not).

The impact of variance in developmental rate on shape

Variation in shape due to developmental timing shows strikingly different patterns in the two species (Table 7). In cotton rats, variation in developmental timing accounts for very little of the variation in shape at any age. It seems to account for a decreasing proportion over ontogeny, but even a drop from the high of approximately 11% to the low of approximately 3% is probably biologically trivial. In striking contrast, in house mice, variance in developmental timing accounts for a sizable proportion of shape variance early in ontogeny and decreases substantially, from nearly 30% in the youngest to approximately 10% in the oldest sample. The transitory low value found in the 15-day-old sample may indicate a temporary reduction in variance, but it could be a fluke of sampling. In either case, it does not appear that the decreasing variance in developmental timing could explain the decreasing variance in shape, because variance in developmental timing and shape variance show different patterns of change. Variance in developmental timing rises from 15 to 20 days and remains high through 25 days, whereas variance in shape is constant.

Table 6. *t* values for the pair-wise comparisons between size variances

Ages	*Sigmodon fulviventer*	Ages	*Mus musculus domesticus*
1–10	2.14	10–15	1.26
10–20	1.04	15–20	0.19
20–30	0.15	20–25	0.11
30–40	2.48	25–30	0.41
40–50	1.92	30–40	0.79
		40–50	0.26

To determine whether the decrease of variance in shape might be caused by the decreasing variability in developmental timing and size, we can look at the dynamics of variance unexplained by either of those factors (Table 8). Clearly, in cotton rats the residual variance shows the same pattern as the total; variance halves between the youngest and next youngest samples and thereafter decreases at a much lower rate (and more episodically). Nevertheless, over the course of ontogeny, variance diminishes to just 37% of its initially high value. In house mice, the dynamics of shape variance are more strongly affected by removing the variance in size and developmental timing. The 15-day-old sample is no longer half as variable as the 10-day-old sample; rather, it has 74% of the variance of the younger age class. And the rate of decrease is smoother; the 20-day-old sample has 79% of the variance of the 15-day-old sample rather than 90%. So rather than an abrupt drop in variance followed by a subtle trend toward decreasing variance, the loss of variance is more

Table 8. The ontogenetic dynamics of the shape variance unexplained by either size or developmental timing

Age	*Sigmodon fulviventer*	*Mus musculus domesticus*
1	1.116	—
10	0.593	0.362
15	—	0.267
20	0.647	0.211
25	—	0.271
30	0.546	0.254
40	0.640	0.210
50	0.411	0.203

Estimates are based on the common subset of landmarks; all values are multiplied by 1000.

evenly distributed across the first 10 days of ontogeny. Notwithstanding this change in dynamics over the course of ontogeny, variance is still reduced to 58% of its initial value between 10 and 20 days and to 56% of its 10-day-old value by 50 days.

DISCUSSION

Variance of skull proportions decreases over ontogeny, confirming results previously reported for cotton rats (Zelditch et al. 1993) and *C. expulsus* (Hingst-Zaher et al. 2000). In these sigmodontine species, and also in the murid house mouse, variance exhibits a similar and striking temporal pattern: It halves early in postnatal growth and thereafter is nearly constant. The reduction in variance does not appear to result from a more general regulation of skull size or developmental timing. In both species, size does appear to be regulated; house mice follow the temporal pattern described for laboratory rats (Nonaka and Nokata 1984)—a trend toward decreasing variance before weaning and then a transitory increase, succeeded by stability. Cotton rats follow a different pattern—a decrease after eye opening and another preceding sexual maturity. But the regulation of size cannot explain the decreasing variance in shape for two reasons. First, variation in size never accounts for more than 12% of the variation in shape with the exception of the eldest sample of *S. fulviventer*, and, second, even after statistically removing the variance due to size, shape variance halves.

Variation in degree of maturity has a pronounced impact on variation in skull shape in house mice but not in cotton rats. Although two-species comparisons must be interpreted with caution (Garland and Adolph 1994), the striking difference between species in the proportion of variance explained by developmental timing in the youngest cohorts may reflect their different life-history strategies. That variation of developmental timing of neonatal cotton rats is so low, and that it does *not* decrease ontogenetically, may indicate that cotton rats are regulating neonatal maturity. Unlike deviations around the mean trajectory of shape, those lying along it would not tend to accumulate over time because everyone eventually matures; because developmental rates are much higher early in development (Zelditch et al. 2003), being immature when young has a larger impact on shape than does being immature later in development. For example, an individual cotton rat that is less mature than the norm by 1 day differs from the 1-day-old mean by 3% (in degree of progress toward adult maturity) but 2% from the 35-day-old mean, falling to 1% from the 50-day old mean.

Variation in shape unrelated to developmental timing is likely to accumulate over time because there are many directions in which variation is possible; being deviant in one direction at one age neither compensates for, nor precludes, being deviant in another direction at another age. For example, having an unusually long incisive foramen relative to palatal length at birth does not compensate for, nor preclude, deviating in another direction at another age (e.g., acquiring an unusually wide cranial base relative to cranial length). Additionally, different individuals can deviate in different directions; that is, one can be unusually wide in one region, whereas a different one is unusually wide in another. Unless variation is removed as rapidly as it is generated, newly arising variation will add to that persisting from an earlier age. Our results are consistent with two scenarios: (a) variation generated during fetal or early postnatal growth is not immediately compensated and therefore accumulates, whereas later, variation is continually generated and immediately compensated, or (b) variation generated during fetal and early postnatal growth is rapidly compensated, after which no new variance is produced. The first hypothesis proposes that compensation is delayed if the variation arises early but not later, requiring an explanation for this temporal asymmetry, whereas the second proposes that processes of skeletal development are buffered so no variance is generated in the first place after (approximately) the eruption of the first molar.

One possible explanation for the high variance of the youngest samples is preservational artifacts engendered by measuring small, fragile, and malleable infant skulls. The small size of the infant skulls is not likely to inflate variance because neonatal cotton rats are not small in comparison with house mice—their skulls are as large as those of 30-day-old house mice. The malleability of the infant skulls does make them prone to deformation, and their fragility makes them prone to damage, but we doubt that artifacts explain the high variance of the youngest samples, for three major reasons. First, among the most variable landmarks are those along the midline of the skull, which are least likely to be affected by deformation. Second, the variable lateral landmarks are

consistently variable across ontogeny. And third, several of the most problematic landmarks are omitted from the subset of landmarks common to both species, but variance still halves. Another possible explanation for the decrease in variance is decreasing measurement error. Although it is no more difficult to locate or digitize the landmarks on images of the youngest specimens than on the oldest, it may be more difficult to orient the youngest skulls consistently. This possibly needs to be examined more carefully, but inconsistencies in orientation might be expected to affect all the landmarks, and several do not show striking or rapid decreases in variance.

The most likely explanation for the high variance found early in postnatal ontogeny lies in the soft tissues directing bone growth. Bone is responsive to forces imposed on the skull, such as those due to contracting muscles and the expansion of the brain (Moss and Salentijn 1969; Wolpert 1981; Carter 1987; Skerry 2000). When dynamically loaded, bone cells undergo a cascade of responses, although the initial stage is not yet understood; the experimentally ascertained sequence of reactions involves an increase in intracellular osteoblast Ca^{++} and then an increase in protein kinase C activation, followed by expression of genes (including transforming growth factor β and insulin-like growth factor I) that is succeeded by osteoblast proliferation and matrix synthesis, resulting in mineralization (Skerry 2000; Yu et al. 2001; Fong et al. 2003). Even prenatally, muscle loading is required for normal development, as evident in the abnormalities of neonatal skulls resulting from mutations affecting muscle development (Herring and Lakars 1981). Resorption is also a critical part of the process as documented by the pathologies of skulls developing without functional osteoclasts (Grüneberg 1936, 1963). From what is known about the response of bone to physical forces, it is likely that these play a role in shaping and remodeling the skull. But it is unlikely that these directly canalize skull shape; according to one model, which is still admittedly controversial, it is not the bony phenotype but rather strain, measured by a ratio between the deformation induced by a force relative to the original dimension, that is regulated. Although strains vary across bones and ages, as do responses to strain (Lieberman et al. 2003), strains for particular bones appear to be maintained at nearly constant levels by the balance between deposition and resorption (Frost 2000; Skerry 2000).

The regulation of strain can explain why parts of an individual's skull might be adapted to the forces imposed on them but does not explain why variance in proportions would initially be high then rapidly fall. One possible explanation lies in the mechanical signals being transduced. Ontogenetic studies of function indicate that prenatal and early postnatal muscular movements are relatively disorganized (Wineski and Herring 1984; Westneat and Hall 1992; Green et al. 1997). They have even been characterized as "fidgety," fine-tuning over time (Forssberg 1999). Variance may arise when growing bone is responsive to disorganized signals. Maturation of neuromuscular control, and also of the brain and sensory organs whose growth exerts tensile forces on the neurocranium, may (rapidly) reduce the variance in shape. This hypothesis implies that voluntary behavior is a component of the epigenetic interactions shaping skull form, a hypothesis supported by a surprising result found in a comparison of deer mice (*Peromyscus maniculatus bairdii*) fed hard and soft diets (Myers et al. 1996). It was anticipated that dietary consistency would affect skull shape because of the stronger forces required for chewing hard food, but several of the anticipated differences were not found, most notably in palatal shape. That discrepancy was explained by the tendency of the mice to gnaw the bars of their cages, compensating for the lower loads experienced during chewing food.

Our explanation for the regulation of skull shape implies that shape itself is not being regulated, rather localized forces are. However, there is a reciprocal interaction between the ontogeny of shape and physical forces because changes in bone shape alter directions of muscle force, thereby altering directions in which bones are loaded and loading influences directions of growth (Herring 1985; Langenbach and van Eijden 2001). Our explanation for the regulation of skull shape also implies that skull shape is canalized because of, rather than despite, the plasticity of bone. But we do not mean to suggest that skull shape itself is unregulated—any disruptions of normal spatial patterning early in development would have profound consequences for shape far beyond the range of variation found in our samples. Those deviations might be either barred by generative constraints or eliminated by stabilizing selection before birth. Nor do we mean to deny the role that genes play in shaping the skull and its variability; rather, we are placing the factors that regulate their expression and also their products in epigenetic context. It would be interesting to examine the dynamics of variance around a pathological norm, such as that produced by mutations affecting muscle development, osteoblasts and osteoclasts, or the surgical removal of muscles. We anticipate that mutations precluding musculoskeletal interactions ought to curtail the reduction of variance and to a greater extent than those affecting overall growth.

Our hypothesis for the regulation of shape variance does not fully explain why fluctuating asymmetry of skeletal morphology would increase even as variance decreases. Whether this contrast between their temporal patterns is a general phenomenon is still unclear, however, given that both variance and fluctuating asymmetry of limbs decrease prenatally in random-bred mice (Hallgrímsson et al. 2003). But even if the temporal patterns are similar, the spatial patterning of these components of variation can differ (Debat et al. 2000; Klingenberg et al. 2002). Nevertheless, our

hypothesis for the regulation of variance, taken together with Hallgrímsson's (1998, 1999) hypothesis for the causes of fluctuating asymmetry, imply that they have a common developmental origin. Just as his morphogenetic drift hypothesis implicates asymmetric musculoskeletal interactions as the cause of fluctuating asymmetry, we point to the organization of musculoskeletal, neuromuscular, and neuroskeletal interactions as the cause of developmental compensation. Consequently, both fluctuating asymmetry and the uncanalized variance are caused by disorganized (including asymmetric) interactions between soft tissues and associated bones.

Having focused on the developmental evidence for canalization, we cannot explain why cotton rats are twice as variable as house mice. *Calomys expulsus* seems to be comparable with cotton rats (Hingst-Zaher et al. 2000; variances recalculated according to the formula used herein). Possibly, all three species are equally well canalized if measured in their natural environments, which is the laboratory for the ICR strain of house mice but not for wild-caught cotton rats and *C. expulsus*. Possibly, the maternal component of variance is disproportionately high in infants of poorly acclimated mothers; the maternal component of variance seems to be generally highest at the youngest ages and, like the genetic component, is reduced over time (Riska et al. 1984), although the pattern varies somewhat over studies and perhaps over species (Atchley and Rutledge 1980 and references cited therein). It may not be necessary to invoke stabilizing selection in the laboratory to explain the lower variance of laboratory mice.

Whether stabilizing selection is necessary to produce canalization is a generally unresolved issue. It has long been assumed that it is, and many studies, both theoretical and empirical, have demonstrated that stabilizing selection can reduce variance (Waddington 1960; Scharloo et al. 1967; Gavrilets and Hastings 1994; Stearns et al. 1995; Wagner et al. 1997). However, networks of coupled genes also can (Siegel and Bergman 2002), and networks of coupled epigenetic processes might similarly do so even late in development.

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REFERENCES

Atchley, W. R., and Rutledge, J. J. 1980. Genetic components of size and shape. I. Dynamics of components of phenotypic variability and covariability during ontogeny in the laboratory rat. *Evolution* 34: 1161–1173.

Carter, D. R. 1987. Mechanical loading history and skeletal biology. *J. Biomech.* 20: 1095–1109.

Debat, V., Alibert, P., David, P., Paradis, E., and Auffray, J.-C. 2000. Independence between developmental stability and canalization in the skull of the house mouse. *Proc. R. Soc. Lond. B* 267: 423–430.

Dryden, I. L., and Mardia, K. V. 1998. *Statistical Shape Analysis*. John Wiley & Sons, New York.

Fong, K. D., et al. 2003. Equibiaxial tensile strain affects calvarial osteoblast biology. *J. Craniofac. Surg.* 14: 348–355.

Forssberg, H. 1999. Neural control of human motor development. *Curr. Opin. Neurobiol.* 9: 676–682.

Frost, H. M. 2000. The Utah paradigm of skeletal physiology: an overview of its insights for bone, cartilage and collagenous tissue. *J. Bone Miner. Metab.* 18: 305–316.

Garland, T., Jr., and Adolph, S. C. 1994. Why not to do two species comparative studies: limitations on inferring adaptation. *Physiol. Zool.* 67: 797–828.

Gavrilets, S., and Hastings, P. 1994. A quantitative-genetic model for selection and developmental noise. *Evolution* 48: 1478–1486.

Goodall, C. 1991. Procrustes methods in the statistical analysis of shape. *J. R. Stat. Soc. Ser. B* 53: 285–339.

Green, J. R., Moore, C. A., Ruark, J. L., Rodda, P. R., Morvee, W. T., and Van Witzenburg, M. 1997. Development of chewing in children from 12 to 48 months: longitudinal study of EMG patterns. *J. Neurophysiol.* 77: 2704–2716.

Grüneberg, H. 1936. Grey-lethal, a new mutation in the house mouse. *J. Hered.* 27: 105–109.

Grüneberg, H. 1963. *The Pathology of Development*. Blackwell, Oxford.

Hall, B. K., and Miyake, T. 1995. How do embryos measure time? In K. J. McNamara (ed.). *Evolutionary Change and Heterochrony*. John Wiley & Sons, New York, pp. 708–720.

Hallgrímsson, B. 1998. Fluctuating asymmetry in the mammalian skeleton: evolutionary and developmental implications. *Evol. Biol.* 30: 187–251.

Hallgrímsson, B. 1999. Ontogenetic patterning of skeletal fluctuating asymmetry in rhesus macaques and humans: evolutionary and developmental implications. *Int. J. Primatol.* 20: 121–151.

Hallgrímsson, B., Miyake, T., Wilmore, K., and Hall, B. K. 2003. Embryological origins of developmental stability: size, shape and fluctuating asymmetry in prenatal random bred mice. *J. Exp. Zool. Mol. Dev. Evol.* 296B: 40–57.

Herring, S. W. 1985. The ontogeny of mammalian mastication. *Am. Zool.* 25: 339–349.

Herring, S. W., and Lakars, T. C. 1981. Craniofacial development in the absence of muscle contraction. *J. Craniofac. Genet. Dev. Biol.* 1: 341–357.

Hingst-Zaher, E., Marcus, L., and Cerqueira, R. 2000. Application of geometric morphometrics to postnatal size and shape changes in the skull of *Calomys expulsus*. *Ital. J. Mammal.* 11: 99–113.

Klingenberg, C. P., Barluenga, M., and Meyer, A. 2002. Shape analysis of symmetric structures: quantifying variation among individuals and asymmetry. *Evolution* 56: 1909–1920.

Langenbach, G. E. J., and van Eijden, T. M. G. J. 2001. Mammalian feeding motor patterns. *Am. Zool.* 41: 1338–1351.

Levene, H. 1960. Robust tests for equality of variances. In I. Olkin, S. G. Ghurye, W. Hoefding, W. G. Madow, and H. B. Mann (eds.). *Contributions to Probability and Statistics*. Stanford University Press, Stanford, pp. 278–292.

Lieberman, D. E., Pearson, O. M., Polk, J. D., Demes, B., and Crompton, A. W. 2003. Optimization of bone growth and remodeling in response to loading in tapered mammalian limbs. *J. Exp. Biol.* 206: 3125–3138.

Mathworks. 2000. *MATLAB*. The Mathworks, Natick, MA.

Miyake, T., Cameron, A. M., and Hall, B. K. 1996. Detailed staging of inbred C57BL/6 mice between Theiler's [1972] stages 18 and 21 (11–13 days of gestation) based on craniofacial development. *J. Craniofac. Genet. Dev. Biol.* 16: 1–31.

Monteiro, L. S., and Falconer, D. S. 1966. Compensatory growth and sexual maturity in mice. *Anim. Prod.* 8: 179–192.

Morgan, T., Garland, J. T., and Carter, P. A. 2003. Ontogenies of mice selected for high voluntary wheel-running activity. I. Mean ontogenies. *Evolution* 57: 646–657.

Moss, M. L., and Salentijn, L. 1969. The primary role of the functional matrix in facial growth. *Am. J. Orthodont.* 55: 566–577.

Myers, P., Lundrigan, B. L., Gillespie, B. W., and Zelditch, M. L. 1996. Phenotypic plasticity in skull and dental morphology in the Prairie Deer Mouse (*Peromyscus maniculatus bairdii*). *J. Morphol.* 229: 229–237.

Nonaka, K., and Nakata, M. 1984. Genetic variation and craniofacial growth in inbred rats. *J. Craniofac. Genet. Dev.* 4: 271–302.

Riska, B., Atchley, W. R., and Rutledge, J. J. 1984. A genetic analysis of targeted growth in mice. *Genetics* 107: 79–101.

Rohlf, F. J. 1998. *TPSRegr*. State University of New York, Stony Brook.

Rohlf, F. J., and Slice, D. 1990. Extensions of the Procrustes method for the optimal superimposition of landmarks. *System. Zool.* 39: 40–59.

Scharloo, W., Hoogmoed, M. S., and Kuile, A. T. 1967. Stabilizing and disruptive selection on a mutant character in *Drosophila*. I. The phenotypic variance and its components. *Genetics* 56: 709–726.

Shalitin, S., and Phillip, M. 2003. Role of obesity and leptin in the pubertal process and pubertal growth—a review. *Int. J. Obes.* 27: 869–874.

Siegal, M. L., and Bergman, A. 2002. Waddington's canalization revisited: developmental stability and evolution. *Proc. Natl. Acad. Sci. USA* 99: 10528–10532.

Skerry, T. 2000. Biomechanical influences on skeletal growth and development. In P. O'Higgins and M. J. Cohn (eds.). *Development, Growth and Evolution: Implications for the Study of the Hominid Skeleton*. Academic Press, New York, pp. 29–39.

Stearns, S. C., Kaiser, M., and Kawecki, T. J. 1995. The differential genetic and environmental canalization of fitness components in *Drosophila melanogaster*. *J. Evol. Biol.* 8: 539–557.

Tanner, J. M. 1963. Regulation of growth in size in mammals. *Nature* 199: 845–850.

Van Valen, L. 1978. The statistics of variation. *Evol. Theory* 4: 33–43.

Waddington, C. H. 1942. Canalization of development and the inheritance of acquired characters. *Nature* 150: 563–565.

Waddington, C. H. 1952. Canalization of the development of a quantitative character. In C. H. Waddington and E. C. R. Reeve (eds.). *Quantitative Inheritance*. Her Majesty's Stationary Office, London, pp. 43–46. Reprinted in C. H. Waddington, 1975. *Evolution of an Evolutionist*. Cornell University Press, Ithaca, pp. 98–103.

Waddington, C. H. 1960. Experiments on canalising selection. *Genet. Res.* 1: 140–150.

Wagner, G. P., and Misof, B. Y. 1993. How can a character be developmentally constrained despite variation in developmental pathways? *J. Evol. Biol.* 6: 449–455.

Wagner, G. P., Booth, G., and Bagher-Chaichian, H. 1997. A population genetic theory of canalization. *Evolution* 51: 329–347.

Westneat, M. W., and Hall, W. G. 1992. Ontogeny of feeding motor patterns in infant rats: an electromyographic analysis of suckling and chewing. *Behav. Neurosci.* 106: 539–554.

Wineski, L. E., and Herring, S. W. 1984. Postnatal development of masticatory muscle function. *Am. Zool.* 24: 1009.

Wolpert, L. 1981. Cellular basis of skeletal growth during development. *Br. Med. Bull.* 37: 215–219.

Yu, J. C., Lucas, J. H., Fryberg, K., and Borke, J. L. 2001. Extrinsic tension results in FGF-2 release, membrane permeability change, and intracellular Ca++ increase in immature cranial sutures. *J. Craniofac. Surg.* 12: 391–398.

Zelditch, M. L., Bookstein, F. L., and Lundrigan, B. L. 1992. Ontogeny of integrated skull growth in the cotton rat *Sigmodon fulviventer*. *Evolution* 46: 1164–1180.

Zelditch, M. L., Bookstein, F. L., and Lundrigan, B. L. 1993. The ontogenetic complexity of developmental constraints. *J. Evol. Biol.* 6: 121–141.

Zelditch, M. L., Lundrigan, B. L., and Sheets, H. D. 2003. Do precocial mammals have a faster developmental rate? A comparison between *Sigmodon fulviventer* and *Mus musculus domesticus*. *J. Evol. Biol.* 16: 708–720.