

The ontogenetic complexity of developmental constraints

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Abstract

Developmental constraint is a theoretically important construct bridging ontogenetic and evolutionary studies. We propose a new operationalization of this notion that exploits the unusually rich measurement structure of landmark data. We represent landmark configurations by their partial warps, a basis for morphospace that represents a set of localized features of form. A finding of developmental constraint arises from the interplay between age-varying means and age-specific variances in these subspaces of morphospace. Examination of variances and means in 16 ventral skull landmarks in the cotton rat *S. fulviventor* at ages 1, 10, 20, and 30 days yielded three types of developmental constraint: canalization (constraint to relatively constant form age by age); chrcods (reduction of variance orthogonal to the mean trajectory over ages); and opposition (reduction of age-specific variance *along* the mean trajectory over ages). While canalization and chreodic constraints have been noted previously, the oppositional type of constraint appears novel. Only one of our characters, relative length and orientation of the incisive foramen, appears to be canalized. Although skull growth becomes increasingly integrated through ontogeny, our characters display a remarkable spatiotemporal complexity in patterns of variance reduction. The specific assortment of constraints observed may be related to the precociality of *Sigmodon*. We suggest that Waddington's diagrammatic presentation of the "epigenetic landscape" may be misleading in quantitative studies of developmental regulation.

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As an infant mammal grows, its skull typically lengthens more than it widens, its face elongates relative to its cranium and, in profile, its cranial base straightens by an increase in the ventral angle between the basioccipital and basisphenoid bones. Also, muscle activity changes ontogenetically, in part because changes in shape of the craniofacial skeleton can alter directions of muscle pull. Like skull shape and biomechanics, developmental integration of the skull has an ontogeny (Zelditch et al., 1992). In general, ontogenetic change is a basic and fundamental feature of phenotypes (Waddington, 1968); the change often involves age-specific phenotypic variance as well as age-specific typical form. Our particular concern in this paper is developmental regulation, the control or decrease of variance of phenotypic features as age increases.

Variance is sometimes reduced ontogenetically by selective death of deviants; in addition, phenotypic variance of some features is also developmentally regulated. For example, despite considerable variation of body weight and rates of weight gain among neonatal ICR randombred rats, the population range of body weights soon narrows as high early growth rate is compensated by early age at puberty (Riska et al., 1984). Variance of inbred mouse skull size also appears to decrease ontogenetically (Nonaka and Nakata, 1984). Variances of barb length and interbarb distance of the feather tip of a warbler, *Phylloscopus inornatus*, also decrease during development (Price et al., 1991). Even in pulmonate snails (*Theba pisana*), not noted for regulatory development, the variability of size measurements and ratios amongst them appear to decrease through ontogeny (Foote and Cowie, 1988).

The most familiar type of developmental regulation is canalization (Waddington, 1939, 1940). Canalization is depicted graphically by what Gilbert (1991) termed “Waddington’s popular icon” – the “epigenetic landscape”. The (metaphoric) landscape, with its hilly terrain and deeply rutted lanes, represents ontogeny as a preferred developmental path downhill, gravitationally stabilized against genetic and environmental factors that might push development of individuals, or individual organs, off course. Combining the Greek words for “necessary” and “path”, Waddington (1961) coined the term “chreod” for these pathways. It is not just the location of the path that is regulated, but also position along the chreod. Should development be pushed off course, it returns not to the point along the chreod from which it was deflected but to a later age-appropriate position. When development is canalized, individuals reach a common end-point despite variation in conditions encountered during ontogeny. Generalizations like these, of course, have the status of definitions, not findings.

Besides canalization, another kind of ontogenetic reduction of variance is the reduction of dimensionality of character covariance matrices – loss of effective degrees of freedom by coordination of variation in characters each still fully variable when observed separately. Both of these models may be required if there is to be adaptive evolution of functional complexes of multiple developmentally individualized parts (Burger, 1986; Wagner, 1988).

In this study, we examine the ontogeny of skull shape variability in the cotton rat *Sigmodon fulviventer* (Muridae) and the spatiotemporal complexity of its growth regulation. Our aim is to distinguish a variety of ways in which variance may be lost

over ontogeny. We examine quantitative aspects of skull shape because, while closely related mammals typically have the same topology of anatomical parts and structures, such as bones and foramina, they commonly differ in quantitative details of form. Skull shape features display several of the patterns of character evolution that have been interpreted as effects of developmental constraints (e.g. Maderson et al., 1982). Some aspects of skull shape are fairly conservative in some lineages but more variable in others, and shape characters display considerable homoplasy in rodents. For example, cranial flexion is a diagnostic feature of a New World muroid, *Microryzomys* (Carleton and Musser, 1989), but varies among subspecies of another New World muroid *Peromyscus maniculatus* (Osgood, 1909) and extent of cranial kyphosis appears to distinguish among related genera of other New World muroids, ichthyomyine rodents (Voss, 1988). This paper is concerned with evidence for spatially complex patterns of developmental regulation during ontogeny. We look for these patterns in intrapopulational variance, interpreted developmentally.

We are studying natural variation, not that induced by experimental manipulation. The literature of responses to experimentally imposed stresses hints at region-specific regulation of skull size variability (e.g. Moore, 1967; Moss 1958; Pucciarelli, 1981; Pucciarelli and Oyenhart, 1987). Typically, responses to environmental or genetic factors vary across the skull. But experimentally imposed stresses often exceed those encountered by natural populations, and their localized effects may bear little relationship to localization of undisturbed growth regulation. Furthermore, and more importantly, it is *variance* we wish to study, not *covariance* with a factor. This localization, however, does not appear to be limited to experimentally manipulated samples. Unmanipulated inbred mice also appear to exhibit localized regulation: for instance, the neurocranium is characteristically less variable than the upper face and mandible, and its level of variability is more stable over ontogeny (Nonaka and Nakata, 1984).

While such findings imply that variance reduction of measures of form may be spatially organized, only recently have coherent methods for localizing morphometric variability been devised (Bookstein, 1989, 1991). Lacking these novel methods, previous studies could only describe features of covariance matrices (such as their principal components) or the "overall" variability of a group of measures that had been construed as forming a morphological complex. However, different parts of a complex may vary at different ages, or among individuals of the same age; the variance "of" the face could be variance of the premaxilla at one time, variance of the maxilla or of the palate at another. Studies of matrices of interlandmark distances by eigenanalysis cannot properly delineate character-specific patterns of variability because they lack a way to localize and compare the variability of separate regions of the form; there is no spatial information encoded in those matrices.

Our emphasis on homology is one hitherto more characteristic of systematics than population biology, despite earlier exhortations to such concern (Riska, 1989). A study of morphometric variability, like any other comparative study, must ensure that the shape characters compared among organisms are shapes of homologous

parts, and that comparisons of variability across ages pertain to homologous features. We use partial warps, components of the thin-plate spline (cf. Bookstein, 1989, 1991), a procedure according with our desire to quantify localized shape variability and compare variabilities of homologous features.

Ideally, to study the ontogeny of variability, we would have analyzed the variation of whole ontogenetic trajectories for all the developmentally individualized features of skull growth of *Sigmodon fulviventer* that were described in our earlier paper, Zelditch et al. (1992). As we do not have longitudinal data, we instead rely upon "circumstantial evidence": comparisons of within-age variability at 1, 10, 20 and 30 days postnatal age. The assumptions that translate these cross-sectional observations into longitudinal findings are those usual in such studies: randomization of form over birth cohort and age of sacrifice, and no selective mortality in the laboratory. We display and classify the modes of change in within-age variability for each character relative to the change in mean form for that character. We use these characters, each one of which is represented by points on a plane, to explore canalization, chreods, and other distinctive forms of developmental regulation.

Materials and methods

Data

The cotton rat, *Sigmodon*, is a relatively precocial (McClure and Randolph, 1980) New World muroid rodent (Carleton and Musser, 1980). Our samples consist of laboratory-reared *S. fulviventer*: 1-day-old ($N = 14$), 10-day-old ($N = 19$); 20-day-old ($N = 17$), and 30-day-old ($N = 19$) individuals from the Michigan State University Museum. The known-age juveniles were bred from wild-caught parents obtained from multiple sites, or from the first two generations of offspring of these wild-caught individuals. Offspring were selected haphazardly for sacrifice. Samples are genetically heterogeneous; differences attributed to effects of age are not confounded with differences between strains.

Specimens skeletonized by dermestid beetles were photographed in ventral view, with the dorsal surface of the molar teeth oriented parallel to the photographic plane. This orientation is left unchanged as the molars erupt. For a general discussion of the role of orientation in the error analysis of points in such images, see Roth (1993). Discrete points, "landmarks" that could be recognized in forms from neonate through adult (Fig. 1), were digitized from these photographs. Although our analysis is thus restricted to this single two-dimensional projection of the three-dimensional form, points were chosen to cover the entire visible surface as closely and evenly as possible.

Shape coordinates (Bookstein, 1986, 1991) were constructed for each landmark with respect to a baseline of two midline points computed by averaging locations of right and left homologs of landmarks *lf* and *Bo* (Fig. 1). The two baseline points are far apart from each other and close to their contralaterals, and were easily located in all specimens. Shape coordinates were then averaged over right and left

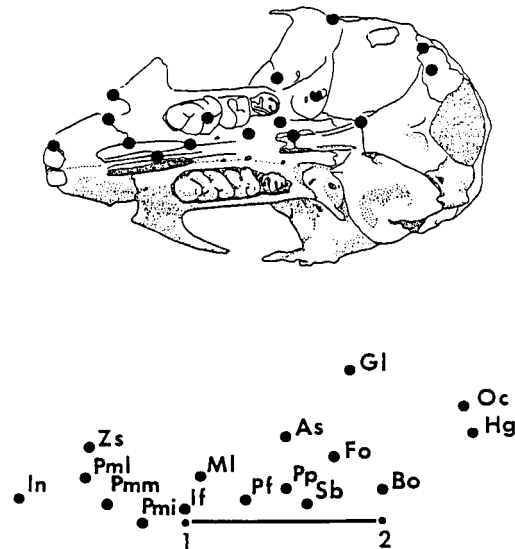


Fig. 1. Landmark locations (for abbreviations and descriptions, see Tab. 1). The baseline points used to construct shape coordinates are 1 (mid-If) and 2 (mid-Bo)

Table 1

Abbreviation	Description
In	Lateral margin of the incisive alveolus, where it intersects the outline of the skull (in the photographic plane).
Zs	Anteriormost point on the zygomatic spine.
Pml	Premaxilla-maxilla suture, where it intersects the outline of the skull (in the photographic plane).
Pmm	Premaxilla-maxilla suture, lateral to the incisive foramen.
Pmi	Suture between premaxillary and maxillary portions of the palatine process.
If	Posteriormost point of the incisive foramen.
Ml	Medium mure of the first molar.
Pf	Posterior palatine foramen (just posterior to the maxilla-palatine suture).
Pp	Posterolateral palatine pit.
As	Junction between squamosal, alisphenoid and frontal; on the squamosal-alisphenoid side of the suture.
Gl	Midpoint along posterior margin of the glenoid fossa.
Fo	Anteriormost point of the foramen ovale.
Sb	Most lateral point on the presphenoid-basisphenoid suture, where it intersects the sphenopalatine vacuity (in the photographic plane).
Bo	Most lateral point on the basisphenoid-basioccipital suture.
Hg	Hypoglossal foramen.
Oc	Juncture between the paroccipital process and mastoid portion of the temporal.

homologs of bilaterally symmetric landmarks. The analyses that follow depend negligibly on this choice of baseline (Bookstein, 1991; Appendix 2; Goodall, 1991).

Methods of shape analysis

We use the thin-plate spline to model shape change as a deformation between landmarks; technical details are supplied in Bookstein (1989, 1991). The method can be explained using a physical metaphor in which relative displacements of landmark points in the (x, y) -plane are depicted as if they were “vertical” – as if transferred to the z -coordinate of an infinite, uniform, infinitely thin metal plate tacked at a given “height” (the “new” form) above the landmarks of an “old” form. The conformation of the surface of this plate is described by a function minimizing physical bending energy, which is a function of the bending (second derivatives) of this artificial z -coordinate. When we instead add the “vertical” to one of the original Cartesian coordinates (x or y), we have a picture of deformation instead of “bending” – a mapping from one picture to another that extends the correspondence to the tissues in between. These interpolations are not intended as models of actual processes acting in-between the landmarks, but only as convenient diagrams expressing features of the statistical space corresponding to the landmark locations, which are the only data. The mathematics of this description can work perfectly well for data in three dimensions, although one’s mental imagery might be stretched by the task of visualizing the resulting six-dimensional “bending”. In this paper we restrict attention to the two-dimensional data from our photographs.

Components of shape change

In the vicinity of a mean or reference form, the thin-plate spline interpolation formula is linear in the “vertical” coordinates – landmark locations in the “target” or “final” form – and bending energy is a quadratic form in those same landmark coordinates (see Bookstein, 1991). This means that we can exploit some familiar matrix algebra to sort out the variety of possible shape variables pertinent to these landmark sets. Closer inspection of bending energy by eigenanalysis of its formula yields two complementary subspaces for the analysis of shape change. One, with no bending energy, describes homogeneous, affine transformations, or *uniform* deformations; the rest of shape space is called the *nonuniform* subspace of transformations. Any deformation is the sum of its uniform and nonuniform components. Likewise, any sample of these skull forms can be treated as a sample of deformations around the mean and thus can be assigned a sample distribution on each of these components.

The *uniform* component describes changes that are geometrically uniform throughout each side of the skull, so that every little square of a starting grid superimposed on the starting form is transformed to the same parallelogram in the same orientation. For these symmetrized skulls in ventral view, the uniform

component of ontogenetic shape change describes elongation (decrease of width relative to length) and shearing (anterior displacement of points proportional to their mediolateral coordinate). There is an age trend of this component (Zelditch et al., 1992). The skull is generally more elongate in older forms, and the more lateral structures, such as the zygomatic arch, are displaced relatively more towards the front of the face than the more medial ones.

Figure 2 shows this mean change as an actual deformation. To visualize the "sample variance" of such an extended geometric abstraction, Bookstein (1991) explains, the shape aspects of a uniform deformation can be reduced to its effect on "one large triangle". In this paper, which uses a midline pair of landmarks as baseline, that uniform part of shape is its effect on an "averaged" lateral landmark, where the averaging is *over landmarks*. We use a coordinate system for which both baseline points, If and Bo, are fixed in position. A sample of deformations is then reducible to a sample of tips of triangles connecting these "typical" landmarks to the If-Bo baseline. We thus arrive at a representation of the means of this "averaged landmark" by age and of interindividual variation around these means in the same two-dimensional plot. Details of this maneuver, which is called the *factor estimate* of the uniform component of a deformation, may be found in Section 7.2 of Bookstein (1991).

The actual ontogenetic shape change of a *Sigmodon* skull is not uniform, but differs from place to place, even in this projected view. We must proceed, therefore,

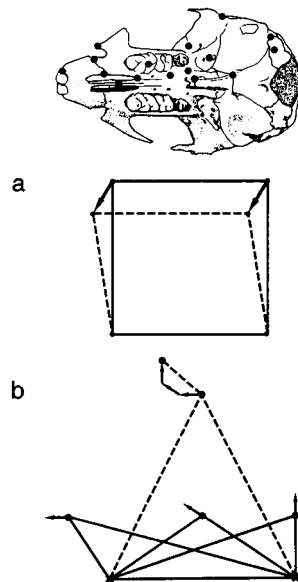


Fig. 2. The uniform component of deformation. a) The deformation: square to parallelogram on either side of the midline. The shear depicted here corresponds to the actual mean change from 1 to 30 days. b) Schematic of the sample uniform shape variance: difference of a generalized "lateral landmark" after restriction of each of the two ends of the baseline.

to describe the residuals from the uniform component. These are the *nonuniform* transformations. When there are more than four landmark points, these can be further decomposed into components (the *partial warps*) ordered by amount of bending energy. Each partial warp may be considered the projection of a surface over the picture, a *principal warp* of the set of landmarks, onto the plane of the picture. The principal warps are eigenfunctions of bending energy that decompose any shape change into an orthogonal set of features. The warps with low bending energy describe large-scale features of shape change – little energy is required to bend the (metaphorical) steel plate between widely separated points. In contrast, relatively more energy is required to bend this metal plate to the same vertical extent between closely spaced points. Thus, the hierarchical ordering of the eigenvectors with respect to bending energy is related to the spatial scales of the corresponding partial deformations. In Zelditch et al. (1992) we give a detailed description of the mean ontogenetic changes of all 13 of these components for our 16 skull landmarks.

One example of a nonuniform component is given in Fig. 3, which shows the largest scale nonuniform component for these landmarks. On the left this transformation is depicted by a pattern of landmark displacements: the most anterior and most posterior points are displaced in the direction opposite to that of the displacements of the middle points. On the right, the same transformation is drawn as a Cartesian transformation of the grid on the left. When the points are displaced in a purely horizontal direction, as in Fig. 3a.1 and 3a.2, this largest scale nonuniform component describes an axial anteroposterior growth gradient – facial elongation with smoothly decelerating growth rates towards the posterior end. When the arrows are oriented vertically, as in Fig. 3b.1 and 3b.2, this describes a relative narrowing of the middle compared to the two ends: in the complete (two-sided) skull, this would be a “pinching” (or the opposite, a central bowing). When oriented at 135° (Fig. 3c.1, 3c.2), this same component reports relative facial elongation and pinching in equal amounts.

Each of these partial warps approximates one of the changes at this scale in our data. The first 10 days of postnatal growth are characterized by almost equal amounts of relative facial elongation and pinching, while the second 10-day interval is characterized by pure relative facial elongation, and the third interval by a small amount of pinching (see, also, Fig. 5, PW1).

The second nonuniform component (Fig. 4, PW2) is at slightly lower spatial scale. Over these 30 days of ontogeny, there is an anteroposterior contraction of the maxillary-palatine-sphenoidal region relative to the premaxilla and occipital (including basioccipital and exoccipitals) and a slight mediolateral narrowing in the diastemal region.

Figure 4 also shows two of the mean ontogenetic changes at still smaller spatial scales. The middle figure (Fig. 4, PW8) depicts the slight elongation and posterolateral rotation of the incisive foramen; the lower figure (Fig. 4, PW10), the lengthening of a small region at the junction of the palatine and sphenoid and the slight widening of the palatine.

Conversion of these partial warps to vectors is easier than conversion of the uniform shears. Each partial warp, although suggesting a deformation, can be

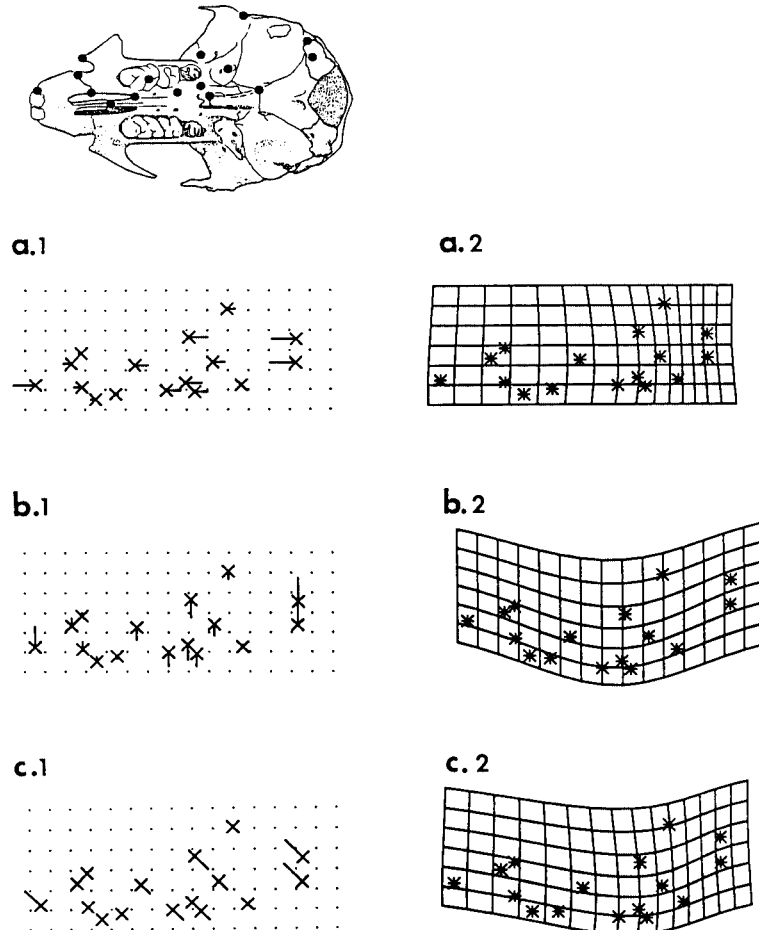


Fig. 3. The largest-scale nonuniform component of transformation. Left, displacement plots (in Procrustes-optimal position); right, deformations. a) The partial warp oriented horizontally. b) The partial warp oriented vertically. c) The partial warp oriented at 135° anterolaterally (proportional to actual change of mean form, 1 to 10 days).

represented as a vector multiple of its own scale-specific principal warp: so much displacement in the x -coordinates of all landmarks, so much in the y -coordinate (Rohlf, 1992). Variation of a sample of these deformations around the age-specific mean deformation may be drawn as a pattern of these vectors around the age-specific mean vector. We have managed, then, to exploit the same diagrammatic style – a mean trajectory, and ellipses around the means – at each geometrical scale. Figure 5 shows the entire set of these diagrams, from the smallest (PW13) through the largest scale of bending (elongation and/or pinching, PW1) to the scale of uniform shear (PW0).

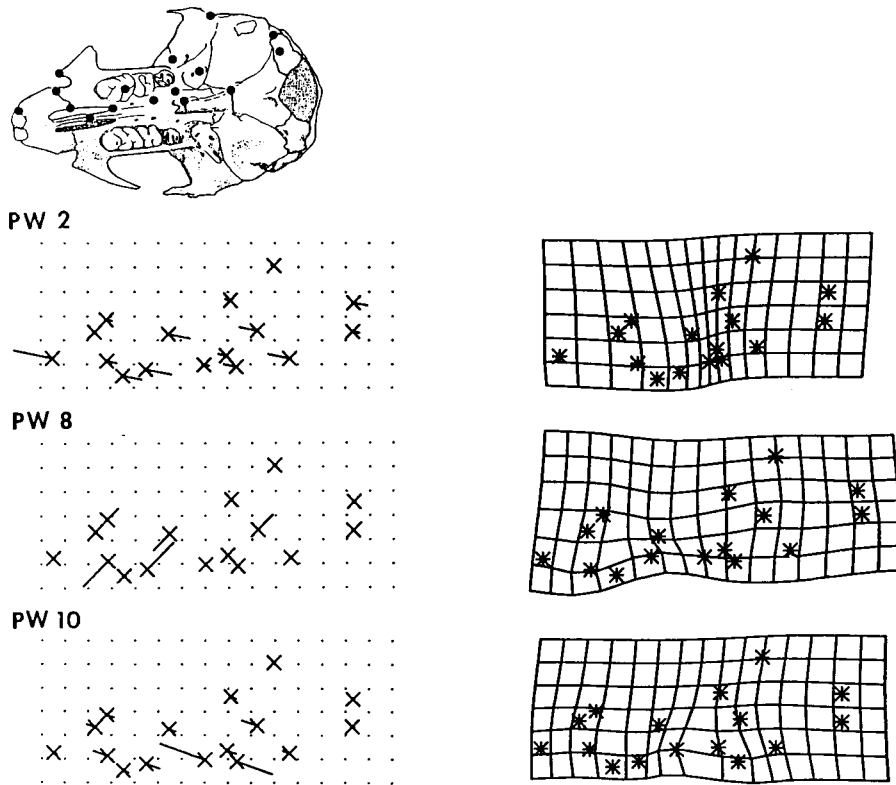


Fig. 4. Some other components of nonuniform variation, 1 through 30 days. Left, displacement plots (in Procrustes-optimal position); right, deformations. a) Second partial warp; b) Eighth partial warp; c) Tenth partial warp. For the sake of legibility, all panels show large extrapolations of the actual change observed (compare Zelditch et al., 1992; Fig. 6)

Our morphometric characters, then, are just like 14 ordinary 2-dimensional variables, the x - and y -components of the partial warp multiplying the uniform component and each of the 13 principal warps. To describe the variability of shape at each scale, we present sample variance ellipses for characters separately, each centered about its own age-specific mean form. With larger samples, we could formally compare these ellipses by relative eigenanalysis. Here we make these comparisons informally, focussing upon the apparent changes of radius of these ellipses (standard deviation of the partial warp “in that direction”) relative to ontogenetic change.

Results

One convenient model of random noise in landmark locations specifies circularity of variance in *each* of these 14 2-dimensional components of shape at each age.

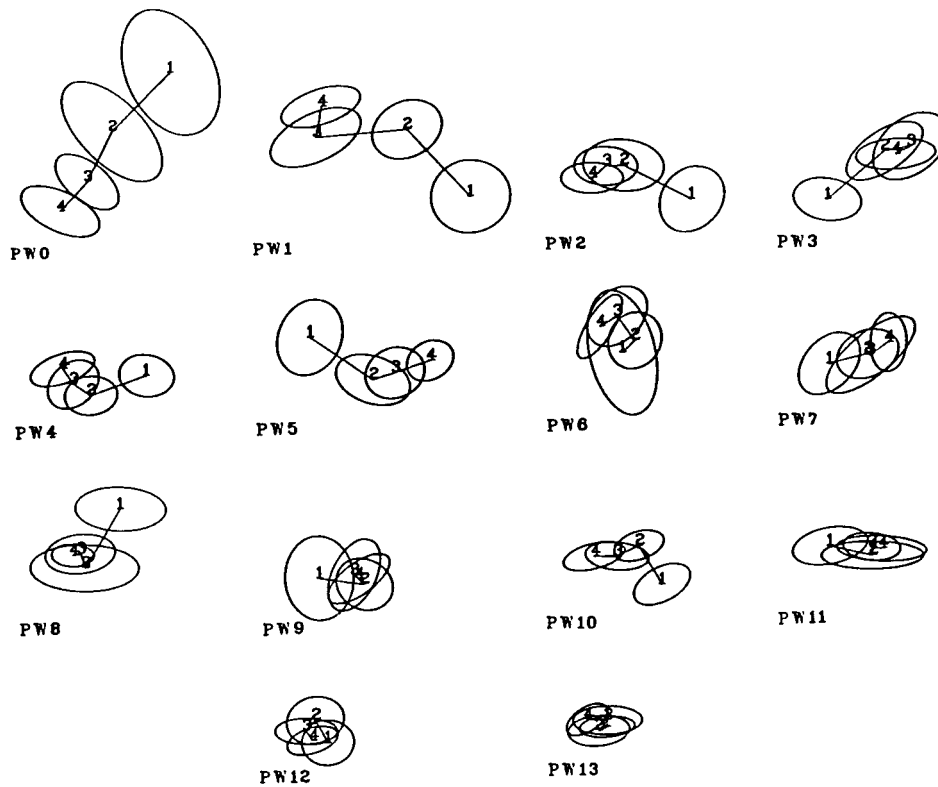


Fig. 5. Superpositions of mean trajectories and age-specific variances in the morphospace of these 16 landmarks: developmental constraint in the subspace of each of the 14 partial warps (0 through 13). The geometry of the starting form is no longer explicit here. Mean trajectories are as in Zelditch et al. (1992), and are drawn to a common Procrustes scale except for PW0. Ellipses represent one standard deviation within age in every direction out from the mean forms. Numbers are located at the age-specific mean (1 = 1 day old; 2 = 10 days old; 3 = 20 days old; 4 = 30 days old). All the pure types of developmental constraint may be found in this figure, and many combinations as well.

While some of our warp scores are circularly distributed, in the data set taken as a whole we find no concentration of evidence in favor of such a model. For instance, variability in uniform skull elongation and shearing is not exactly circular even at birth (Fig. 5, PW0). Over time, the deviation from circularity at this scale increases: specifically, variability in the direction of mean ontogenetic shape change decreases relative to the variability perpendicular to the path. Ideally, we would carry out a statistical test of this observation. But for present purposes, it is sufficient simply to highlight it: The direction of mean ontogenetic change is at the same time the direction of maximal decrease in age-specific variance.

Likewise, for PW1, for the youngest skulls, variability in extent of relative facial elongation and pinching appears to be approximately equal in all directions (Fig. 5,

PW51, ellipse 1). But by 20 days of age (ellipse 3), this variability is no longer circular. It is relatively reduced in a direction at about 25° to the direction of net ontogenetic change (the vector between sample means of 1 and 30 day old forms). Figure 6 (PW1) depicts the direction in which shape variability is maximally constrained as the Cartesian transformation that has apparently been suppressed.

At the next lower spatial scale, the variability of the maxillary-palatine-sphenoidal region that PW2 describes is likewise initially circular (Fig. 5, PW2). By contrast with the two preceding characters, the variance of PW2 is lost preferentially in the direction almost perpendicular to the direction of ontogenetic change (Fig. 6, PW2).

PW3, the component at the next lower spatial scale, describes displacements of Gl, a lateral braincase point, and the anteromedial premaxillary points relative to the points Zs and Pml in-between them (Fig. 1). At this scale (Fig. 5, PW3) variability first increases and then decreases along the direction of net ontogenetic change (Fig. 6, PW3).

The feature at the next smaller spatial scale, when applied in the x -direction, describes variability in extent of elongation of the premaxillary and palatine-sphenoid complex relative to the maxilla and basioccipital. In the y -direction, it specifies extent of relative widening of the premaxilla, anterior maxilla, and occipital region.

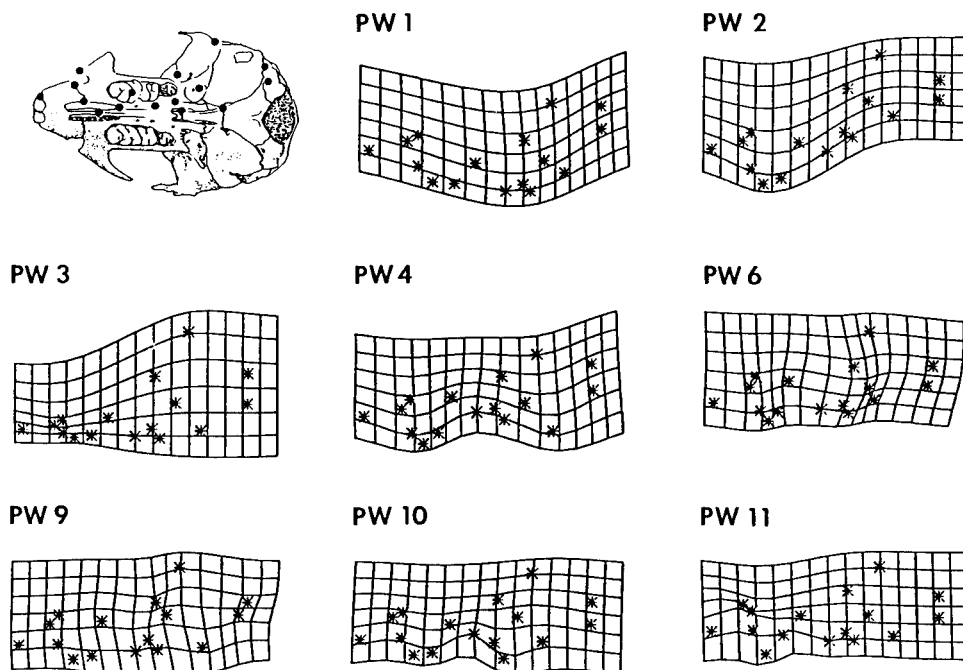


Fig. 6. Partial warps corresponding to apparent directions of relatively constrained variance at 30 days. As in Fig. 4, deformations shown are large multiples of actual sample range.

At birth, variability in this feature (Fig. 5, PW4) appears essentially equal in all directions. From 20–30 days it appears to be reduced in the direction of 10–30 day change in mean form (Fig. 6, PW4).

Features at the next two spatial scales describe integrated variability of points on the lateral braincase and molar landmark. Variability in extent of posteromedial rotation and anteroposterior location of a transect drawn from the molar row to the alisphenoid becomes reduced in the direction perpendicular to the early trajectory (Fig. 5, PW5) and then is lost in the orthogonal direction as well. At a slightly more localized scale, PW6 describes variability in position of the glenoid relative to the alisphenoid, which, ontogenetically, more closely approaches the molar tooth row. Although there is substantial variability of the shape of this region at birth, it is greatly reduced by 10 days, even though the change in average form is quite small. By 30 days of age, shape variability appears greatest in the direction almost perpendicular to the net ontogenetic change (Fig. 6, PW6).

Variability of extent of elongation of the lateral braincase relative to more medial points (Fig. 5, PW7), like PW3 and PW5, initially diminishes in the direction orthogonal to the mean ontogenetic change and then in the perpendicular direction.

The variability of relative length and orientation of the incisive foramen (Fig. 5, PW8) is clearly constrained at birth: There is considerably more variation in the relative length of this foramen than in its orientation. Ontogenetically, the variability is radically reduced at a rate that appears equal in all directions.

The character at the next smaller spatial scale is the displacement of two posteromedial points (the two most posterior foramina) relative to more medial and lateral points. Variance of this character (Fig. 5, PW9) appears to drift ontogenetically; there is no clear relationship between directions of ontogenetic change and variance reduction (Fig. 6, PW9).

Variability of shape of the palatine-sphenoid region (Fig. 5, PW10) appears directional at birth – the more elongated palatines are narrowed with respect to the sphenoid-basisphenoid suture. From 10 days on, variability appears to be preferentially reduced in the direction perpendicular to the mean change of this character (Fig. 6, PW10).

Variability in the relative length and mediolateral position of the posterior portion of the incisive foramen and zygomatic spine is far from circular at birth (Fig. 5, PW11) and is preferentially lost in the direction perpendicular to the ontogenetic trajectory (Fig. 6, PW11).

In this same region, but at a slightly smaller scale, variability in the position of points at the premaxillary-maxillary suture is circular until 20 days (Fig. 5, PW12); After this time, ontogenetic shape change ceases and variability decreases in what was the previous shape trend.

There is no ontogenetic shape change observed at the smallest scale (the posterior portion of the palatine) and no evidence of any ontogenetic trend in variability (Fig. 5, PW13), although the variability of this region is clearly not circular at any age.

We computed the principal components of the within-age variability of the 28 partial warp components separately by age. The ratio of the first two components

does not exceed 1.54 in any of the four samples (1.05 at 1 day; 1.10 at 10 days; 1.26 at 20 days and 1.54 at 30 days). We would not pick out any of these ellipses as significantly noncircular by a test of all 26 components together. That is, we do *not* see any substantial “reduction of dimensionality” of these samples when they are considered in shape space. Only in their natural pairing, combined with features of the mean change, are natural descriptors of developmental regulation generated.

Discussion

Among the features that characterize this configuration of 16 landmarks at each spatial scale, there are changes of mean shape, an ellipse of individual variation around each mean, and changes over ontogeny in that variance. For example, the incisive foramen of the average cotton rat increases in relative length and rotates anterolaterally ontogenetically; at birth there is considerably more variability in the relative length of this foramen than in its orientation, and variance is reduced at the same rate in all directions. Other regions of the skull, while characterized by circular variation at birth (variance indistinguishable from that induced by random digitizing noise), acquire directionality as variance is preferentially reduced along some direction, such as that of the ontogenetic trajectory. The frames of Fig. 5 depicting these changes in mean and in age-specific variance may be directly relevant to a methodological problem in the description of ontogeny, the *developmental constraint*.

We have not seen any rigorous definition of this notion. Gould (1989), for example, characterizes developmental constraint as “a theory-bound term for causes of change and evolutionary direction by principles and forces outside an explanatory orthodoxy . . . compelling or channeling phenotypic change in a direction set by past history or formal structure”. This clearly will prove difficult to attach to any quantification. Some authors define constraints in terms of “limitation(s) on the set(s) of possible developmental states and their morphological expression” (Maderson et al., 1982) rather than in terms of factors channelling evolutionary change. Some authors pursue a more specifically quantitative-genetic definition of constraint, arguing that “quantitative genetic parameters measure the effects of the epigenetic system and developmental constraints on evolutionary response to selection” (Cheverud, 1984).

Several authors have defined developmental constraint in terms of non-random (e.g. Alberch, 1983) or “biased” (e.g. Maynard Smith, 1985) variation. As Wagner (1988) noted, this entails a definition of *unconstrained* variance: a system might be said to be unconstrained when “phenotypic variances and the heritabilities of all the characters are the same and [when] there are neither phenotypic nor genetic covariances among the traits”. Such a definition presupposes a scale by which variances can be found to be “equal” or “unbiased” across diverse characters. In our analysis, the observation of noncircularity is possible only because there is a meaning to circularity for shape space. A notion of circularity, however, presumes a pre-existing metric for all of morphospace. As we have argued elsewhere

(Bookstein et al., 1985; Bookstein, 1991) such a metric seems to be available *only* in the case of coordinate data.

Hence, instead of attempting to sort through the inconsistent definitions of developmental constraint “in general”, we do better to proceed empirically to classify the types that arise in the panels of Fig. 5 – types of constraint as they emerge from the study of coordinate-based morphometric characters. We do not claim that this analysis is optimal in any particular statistical sense. Nevertheless, it seems the first one available, for several reasons. It is necessary that the analysis be carried out explicitly in shape space, without any possibility of confounding by scale (Mosimann, 1970). Any basis for the shape space of a set of landmarks might have been tried; because we wish the greatest conceptual independence across the separate plots of Fig. 5, we have chosen the partial warp basis (rather than, for example, the shape coordinates of the landmarks separately). Furthermore, because they are geometrically localized, these warps are the nearest approximation morphometrics can offer to the systematist’s notion of a “character”. As we are interested in the directionality of the ellipses separately as well as of their changes, we need a natural metric relating variances in the different directions of morphospace. The partial warp scores (and also the shape coordinates) come with such a natural metric, effectively the same as Procrustes distance (Bookstein, 1991; Rohlf, 1992), which supplies a reference “circular” shape to which each of these ellipses can be compared; arbitrary sets of ratios and angles, such as are commonly analyzed in “traditional morphometrics” (Marcus, 1990), do not have access to such a natural metric. Analysis of all the shape coordinates together, in one large principal components analysis, proves unhelpful: the dominant eigenvalues are in the ratio of 4.37:3.67, indicating no particular factor structure at all. In fact, such an analysis blurs distinctions among processes at different scales and in different regions; this would also be the case for analyses of interlandmark distances and of finite elements. When localized analysis is possible, global principal components are not an acceptable substitute. We know of no other form of morphometric analysis that offers a natural metric for comparisons of variance, unbiased by direction, at a variety of geometric scales.

We propose to describe the changes along these series of ellipses using the geometric language of *relative eigenanalysis* (see Bookstein, 1992): rather than describing how the principal axes of the ellipses “rotate”, we instead observe the ratios by which variances in each direction increase or decrease over time. Because our interest is in regulation, i.e. loss of variance, we will emphasize the directions of greatest decrease, whenever we can. In principle, this analysis should be carried out by matrix algebra. We prefer instead to remain at the level of the diagram, and report these changes informally, because our concern is with their qualitative conformity to the current glossary of developmental constraint. At the moment, we are less interested in their standard errors or statistical significance as separate findings.

A variety of “pure types” of these series might be encountered. Each type will pertain to the single two-dimensional character that represents “growth at a particular geometric scale”. Variance might be circular at first, that is, consistent

with random digitizing noise or other circular uncertainty of no obvious factor structure. We see this in the case of PW1 and others as well. Or shape variance at a particular scale might begin elliptical, so that there is a direction of preferred variability or prenatal regulation (as in the case of PW8). Over time, the age-specific variance of one of these characters may remain relatively constant, or it may drop; Figure 5 shows no examples of a systematic or substantial rise. The drop in variance may be more or less isotropic – by the same ratio in every direction – such as we observed for PW8, or that drop in variance may have a preferred direction, as it does for PW1 or PW2. A direction of maximum ratio of decrease may be aligned with the mean trajectory of the character, perpendicular to the trajectory, or oblique to it. If the direction of greatest variance reduction is aligned with the mean trajectory, then the *shorter* principal axis of the variance ellipses will come to lie parallel to that trajectory, as is the case here for PW1 and others. If the direction of greatest variance reduction is perpendicular to the mean trajectory, then the *longer* principal axis of the variance ellipse will come to lie parallel to that trajectory, as is the case for PW2 and others.

Some of these types may correspond to terms current in the literature of developmental regulation. The pattern of a steadily shrinking ellipse, PW8, strikes us as closest to the classic concept of canalization. Over time, this aspect of these *Sigmodon* is being constrained to a nearly constant form within ages, and, whether coincidentally or not, across ages as well. Apparently this is not a common feature of *Sigmodon fulviventer* skull growth, as we see it only once in our set of characters, the relative length and orientation of the incisive foramen. The length and orientation of this foramen, through which pass nasal branches of the palatine arteries and the nasopalatine ducts of the organ of Jacobson (Hull, 1935), vary among muroids, and often have been used as taxonomic characters. No functional significance has been attributed to the variants, but the canalized development at this specific scale suggests that there may indeed be an optimum for this character in this species.

Another type of developmental constraint is the reduction of variance preferentially in the direction perpendicular to the mean trajectory. We see this several times in our data, for PW2, PW10, and PW11. Individuals follow a common developmental sequence along the trajectory, but vary in position along this sequence even within ages. We suggest that this be called *chreodic*, after Waddington's (1961) definition of the "necessary path". It is less appropriate to refer to this as canalization, a word which (in Waddington's glossary) makes no reference to the persistence of variance along the mean trajectory. Hall (1992b) has argued that chreod is an unnecessary term, because "chreods cannot exist without canalization", but this may be because chreods without canalization have not previously been described. It seems important to distinguish loss of variance equally in all directions – canalization – from preferential retention of variance along the mean trajectory. Chreodic growth may facilitate evolutionary change in the direction of ontogenetic change because this is the direction in which there is variance available to select on – descendant taxa would retain ancestral chreods but would differ in age at which primitive morphologies are expressed. The classic concept of heterochrony seems tacitly to assume this scenario, as "changes in developmental rate

and timing” can explain only changes along the typical developmental trajectory, however flexibly.

A third type, possibly the most interesting, seems to have no equivalent in the classic literature of developmental regulation: loss of variance preferentially in the direction of the mean trajectory. We see this in PW0, PW1, PW4, PW12 and perhaps, at later ages, in PW3, PW5 and PW7. Because the major axis of the ellipse is opposed to the trajectory, we suggest labelling this the *oppositional* type of constraint. Oppositional processes exhibit regulation only in the direction of mean change. One may think of the organism as greatly concerned with the effective “age” of that particular shape character, and less concerned to regulate the variance of the character uncorrelated with age. In these characters, there is morphometric evidence neither of chreods nor of any selectively usable variability in rates or timings of progress along the ontogenetic sequence. Oppositional processes may thus impede evolution by heterochrony, as the necessary phenotypic variance is regulated developmentally.

Several of the characters in Fig. 5 cannot be unambiguously assigned to any of these classes. Sometimes there is so little ontogenetic shape change that the orientation of variance cannot reliably be compared to the direction of ontogenetic shape change (e.g. PW13); sometimes the character appears to “drift” without change of variance (e.g. PW9); sometimes the principal direction of variance reduction appears to change from age to age (e.g. PW3, PW5, PW7). These last three characters describe integrated features of the lateral braincase and palate. It is possible that none of these geometric components describe changes effected by any biological process; perhaps they, instead, improperly reify changes in the third dimension (due to basicranial bending) as projected onto a two-dimensional photographic plane. Alternatively, it is possible that there is a transition in biological processes regulating variability common to braincase and palate following cessation of brain growth.

Our characters display a remarkable spatiotemporal complexity in patterns of relative variance reduction. Pooling over scales, we failed to find principal components of variability; the diversity of patterns evident in the 14 separate panels of Fig. 5 suggests that any adequate causal model of growth regulation must have a complex factor-structure, articulated separately at each geometric scale or region. Even such an apparently simple feature as the length of the incisive foramen is developmentally complex. Its description is a summation of geometric components at several scales: changes at the level of the whole skull (PW0, PW1); changes at the level contrasting contraction of the maxilla-palatine-sphenoid region to elongation of the premaxilla and basioccipital (PW2); changes at more localized scales (PW4: PW8); and changes describing relative lengthening of the posterior region of this foramen along with a slight anterolateral displacement of the zygomatic spines (PW11). While any single component might be a compensation for deviations of the processes acting at other scales, the diversity of ontogenetic patterns suggests that each may be separately regulated. Although mean skull growth becomes increasingly integrated through ontogeny (Zelditch et al., 1992), localized growth processes that cease to contribute much to net shape change nevertheless remain

individualized as components of developmental regulation. The description of developmental constraint in skull form is thus far more complex than we had previously thought: There are at least as many types of regulation here as we have types of constraint.

While we doubt that this complexity is a unique feature of *S. fulviventor*, the particular timings and patterns found here may be distinctive. *Sigmodon* is a remarkably precocial muroid. Eye-opening occurs within a day or two of birth; and, furthermore, if disturbed, cotton rats even this young will scatter (McClure and Randolph, 1980). Weaning also occurs at a young age – cotton rats begin eating solid food by as young as 10 days of age, and are weaned, on average, by 15 days (McClure and Randolph, 1980). In comparison, young of other relatively precocial New World muroids, such as *Scotinomys teguina*, open their eyes between 12 and 15 days after birth and are weaned between 18 and 20 days, and the young of *S. xerampelinus* take about 3 days longer to reach the comparable stages (Carleton and Hooper, 1976). Young *Peromyscus maniculatus bairdi*, a relatively precocial *Peromyscus*, open their eyes at 14 days and are weaned by 25 days (Layne, 1968). If the timings of regulation are related to the timings of these developmental stages, rather than to postnatal chronological age, more or less precocial species may differ in temporal aspects of growth regulation, as the stringent regulation of life-history of *S. fulviventor* may entail relatively severe control of any of these features of form age by age.

Directions of variance reduction surely may also vary among taxa. To date, only one other study has examined rodent skull growth using similar geometric methods. Contrasting with the oppositional pattern found here, the variability of the largest (orthocephalic) component of lateral skull form in *Rattus* seems circular at all ages (Bookstein, 1991). Notwithstanding the difference in views of the skull examined, the oppositional pattern may also be due to the precociality of *Sigmodon* development. Precociality in general, and specifically the precociality of *Sigmodon*, may be an adaptive response to high predation pressure on nestlings (Hooper and Carleton, 1976; McClure and Randolph, 1980). Further accelerating independence may incur physiological costs if weanlings achieve independence before they are capable of endogenous heat production. Comparative physiological studies suggest that there may be a minimum time required to develop endogenous heat production (McClure and Randolph, 1980). Young *Sigmodon* achieve endogenous heat production at 12 days, two days after they begin taking solid food and three days, on average, before weaning is complete. In this timing, *Sigmodon* may be close to the lower bound for age at independence. Of the muroids examined, only *Mus* is capable of endogenous heat production at a younger age (11 days). In nature, variability of age at independence may be regulated by selection; our oppositional pattern suggests that developmental age may also be regulated internally.

As all living species of *Sigmodon* are precocial, we might test our biological explanation that highly precocial development is related to the oppositional pattern by a comparative analysis of the geometry of regulation. We cannot simply compare age-specific patterns of shape variability because ontogenetic trajectories vary in their orientation among *Sigmodon* species. For example (Zelditch et al., unpubl.), the global nonuniform component of *S. leucotis* is oriented entirely in the

horizontal direction – midpalatal pinching does not occur during early postnatal growth of *S. leucotis* as it does in other *Sigmodon*. If variance were reduced in the direction of ontogenetic change in *S. leucotis*, this direction of reduction would lie horizontally, and aspects of shape that are most variable would be different in these taxa, even if the processes of developmental constraint was the same in terms of our classification. The combination of common patterns of variance reduction with interspecific variation in ontogenetic trajectories would imply that the type of growth regulation might be a synapomorphy at a more inclusive level than the ontogenies of features being regulated. Systematists are accustomed to finding that a feature regarded as a synapomorphy can be manufactured by varying developmental processes (see Hall, 1992a for examples). The individual parts of the skull, the developmental processes that form these parts, and the processes regulating shape variability may each be a synapomorphy at a different cladistic level. Cladistic studies of developmental regulation, ontogenetic processes, and life-history characters in more ecologically diversified genera (e.g. *Peromyscus*) could examine the congruence and relative lability of these characters.

Our depictions of developmental regulation do not much resemble classic epigenetic landscapes. Waddington's original diagram (1941) showed a ball rolling down a grooved landscape. The intended metaphor of development was precisely of a process "driven" by gravity *both in its chronological dynamics and its stochastic aspects*. Never intended to represent physical reality, these diagrams initially served to unite genetics and embryology by representing effects of variant alleles on development in pictorial terms familiar to embryologists (Gilbert, 1991). But these diagrams fail as heuristics (see Latour, 1989). One who learned to envision developmental regulation according to Waddington's picture will thereafter think of variation perpendicular to the "canal" as *rolling uphill*, as energetically unfavorable. The Waddington diagram, then, implicitly confounds two aspects of variation that are algebraically and empirically separate: the direction of mean change, and the direction of least variation at a given age.

We suggest that our ability to visualize developmental regulation has been inadvertently impoverished by this incomplete visual vocabulary, one so intimately tied to the language of canalization. The diagrams of Fig. 5, while not so evocative, clarify this separation of dimensions by the difference in the two graphical styles. Mean changes are line segments in morphospace, not "height", and variance reduction is narrowing of ellipses, not a redundant use of the same "height". Oppositional regulation may have been neglected before in part because there was no pictorial way to look for it. Here we may have a peculiarly literal case of what Wittgenstein (1958) argued by metaphor: "A *picture* held us captive. And we could not get outside it, for it lay in our language and language seemed to repeat it to us inexorably".

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