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THE RELATIONSHIP BETWEEN FLUCTUATING ASYMMETRY AND ENVIRONMENTAL VARIANCE IN RHESUS MACAQUE SKULLS

KATHERINE ELIZABETH WILLMORE,[1,2] CHRISTIAN PETER KLINGENBERG,[3,4] AND BENEDIKT HALLGRÍMSSON[5,6]
[1]*Department of Medical Sciences, Health Sciences Center, University of Calgary, 3330 Hospital Drive NW, Calgary, Alberta T2N 4N1, Canada*
[2]*E-mail: kewillmo@ucalgary.ca*
[3]*Faculty of Life Sciences, University of Manchester, Michael Smith Building, Oxford Road, Manchester M13 9PT, United Kingdom*
[4]*E-mail: cpk@manchester.ac.uk*
[5]*Department of Cell Biology and Anatomy, Health Sciences Center, University of Calgary, 3330 Hospital Drive NW, Calgary, Alberta T2N 4N1, Canada*
[6]*E-mail: bhallgri@ucalgary.ca*

Abstract.—This study measures the correlation between within- and among-individual variance to gain a greater understanding of the relationship of the underlying mechanisms governing developmental stability and canalization. Twenty-six landmarks were digitized in three dimensions from the crania of 228 adult macaques from Cayo Santiago. The phenotypic variance between individuals was measured and divided into its genetic and environmental components using matriline information. Within-individual variance was measured as the fluctuating asymmetry between bilateral landmarks. We found positive and significant correlations between the phenotypic, environmental, and fluctuating asymmetry variances for interlandmark distances. We also found low but significant correspondences between the covariation structures of the three variability components using both Procrustes and interlandmark distance data. Therefore, we find that in macaque skulls traits that exhibit greater levels of asymmetry deviations also exhibit greater levels of environmental variance, and that the covariances of absolute symmetry deviations partly correspond to covariances of mean deviations at the individual level. These results suggest that the underlying processes that determine canalization and developmental stability are at least partly overlapping. However, the low correlations reported here are also evidence for a degree of independence between these variability components.

Key words.—Canalization, environmental variance, fluctuating asymmetry, *Macaca mulatta*, morphometrics, quantitative genetics.

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Canalization and developmental stability are patterns of phenotypic variability. Both reflect the tendency for development to follow a preferred trajectory toward some phenotypic outcome (Hallgrímsson et al. 2002). These two components of variability have traditionally been distinguished by the origin of the perturbations that they buffer. Waddington (1957) defined canalization as the property of an organism that buffers development against environmental and genetic perturbations to produce a consistent phenotype. Schmalhausen (1949) independently developed a similar concept that he termed "autonomization." In contrast, developmental stability arises from processes that buffer development against developmental accidents that occur under constant environmental and genetic conditions (Waddington 1957). More recently, the distinction between canalization and developmental stability has been reinforced by Zakharov (1992) and Clarke (1993, 1998). Waddington (1957) argued that different underlying processes governed canalization and developmental stability. While it is clear that canalization and developmental stability are different patterns of phenotypic variability, it is not at all clear to what extent variation among and within populations is determined by a distinct or overlapping set of developmental processes.

The empirical and experimental evidence for the relationship between developmental stability and canalization is contradictory. Several studies have shown that there is no association between fluctuating asymmetry (FA) and phenotypic variance, supporting the view that developmental stability and canalization are determined by separate mechanisms (Waddington 1957; Rutherford and Lindquist 1998; Debat et al. 2000; Milton et al. 2003; Réale and Roff 2003). In contrast, other studies have shown correspondence between FA and phenotypic variance (Clarke 1993, 1998; Klingenberg and McIntyre 1998; Woods et al. 1999; Hallgrímsson et al. 2002). These conflicting results illustrate our continued lack of understanding of the processes that govern variability and highlight the need for further research.

The aim of this study is to determine whether there is congruence between FA, environmental, and phenotypic variation in adult macaque (*Macaca mulatta*) crania. FA refers to small deviations from symmetry and is commonly assumed to be an inverse measure of developmental stability. The degree of canalization of particular traits can be measured by the amount of phenotypic variation, which can be further broken down into its environmental and genetic components. We hypothesize that levels of FA and environmental variance (V_E) will be positively correlated. Our hypothesis is consistent with the view that both developmental stability and canalization emerge as by-products of regulatory complexity and redundancy in developmental systems (Siegal and Bergman 2002) and as such share overlapping developmental bases. Our hypothesis is also in agreement with the theoretical model developed by Klingenberg and Nijhout (1999). They found that the nonlinear nature of the genetic variation of trait development was sufficient to cause FA as a result of random perturbations of nongenetic origin. That is, FA can have a heritable component without the need for specific FA genes. If developmental stability arises as epiphenomenon from ge-

neric properties of development, such as nonlinear relationships among components of regulatory networks, it seems unlikely that some properties of developmental-genetic architecture would buffer among-individual variation only while others buffer within-individual variation only. Perturbations that influence different components of developmental systems are likely to be buffered by the developmental organization relevant to each component. However, this does not imply that developmental systems distinguish between perturbations that produce among-individual versus within-individual variation. To the extent that stability arises from generic aspects of developmental organization, it is reasonable to expect that these components of variability share overlapping developmental bases.

Our study expands on existing work in this area in three ways. Based on a large sample of rhesus macaques of known maternal relationship, we partition the phenotypic variance into genetic, environmental, and FA components. This expands on our earlier study of mouse fetal limb measurements in which we found a positive association between univariate FA and V_E (Hallgrímsson et al. 2002). In that study, the estimates of genetic variances are problematic because they were based on within-litter variances and so confounded by the among-litter environmental variance. Secondly, we used cranial traits rather than limb elements. It has been suggested that characters related to locomotion would have greater symmetry than characters that are not associated with locomotion, as they may be more important for fitness (Debat et al. 2000). While there is no empirical evidence for this claim, it is of interest to see how patterns of variability compare between cranial and postcranial elements. Finally, this study uses both univariate and multivariate approaches to compare variance components among traits as well as covariance structures among variance components. These two approaches measure different things. For the univariate analysis, covariation among FA and among-individual variances is produced when traits or samples with high FA also have high among-individual variation. We predict that if there is overlap in the underlying developmental basis of developmental stability and canalization, then traits that express greater levels of FA will likewise express greater levels of environmental variance. In the multivariate analysis, correlations among the phenotypic, genetic, and environmental variance/covariance matrices and the asymmetry variance/covariance matrix are produced when the covariances of individual-level mean deviations are related to the covariances of the absolute asymmetry deviations. In other words, the multivariate approach quantifies the degree to which the concordance of mean deviations corresponds to the concordance of asymmetry deviations. Both approaches quantify the effects of the underlying relationship between developmental stability and canalization, but they do not necessarily quantify the same results of that relationship.

Materials and Methods

Composition of the Sample

The sample consists of 228 adult *M. mulatta* macerated crania. The macaques used in this study are from Cayo Santiago, a small island off the coast of Puerto Rico. This population was introduced to the island in 1938 and has since survived under semi-free-ranging conditions (Rawlins and Kessler 1986, pp. 47–48). The macaques forage on the available tropical vegetation and are also given daily provisions of commercial monkey chow. Water is provided ad libitum (Rawlins and Kessler 1986, pp. 47–48). Matriline information is known for each individual allowing the estimation of the heritability of different traits. A total of 96 males and 132 females were used in this study, all of them adults. Choice of individuals was based on the completeness/lack of damage to the skull and the number of related adult individuals.

Data Collection

The data consist of 24 pairs of bilateral landmarks and two midline landmarks recorded in three dimensions (see Fig. 1). In addition to being highly repeatable, these landmarks encompass elements of the entire cranium. Measurements were digitized using the three-dimensional Microscribe (Immersion Corp., San Jose, CA) and were directly recorded as x-, y-, and z-coordinates.

The skulls were held in place using modeling clay, and were positioned so that all of the landmarks could be digitized without repositioning the specimen during measurement. To determine measurement error, each individual was measured three times by the same observer (K. E. Willmore) on separate days.

The landmark data was used to calculate Euclidean distances. Of the possible distances among the landmarks, 60 were chosen because they outlined anatomical regions of interest. These distances were computed for both sides of the skull; 34 of them were ultimately used in this study, as they showed significant FA over measurement error (see Table 1). Moreover, the landmark coordinates were used directly for shape analyses using the methods of geometric morphometrics.

Data Analysis

Several methods are used to quantify morphometric data, and the choice of these methods is controversial. This study employs traditional morphometric analyses using Euclidean distance data, as well as geometric morphometric analyses using Procrustes superimposed data. Both methods offer advantages and limitations, and we attempt to exploit the positive attributes while minimizing the potential pitfalls associated with both methods by using a battery of analyses. Geometric morphometrics using Procrustes superimposed data offers a convenient method to visualize overall shape variation (Richtsmeier et al. 2002; Zelditch et al. 2004a, p. 124; Hallgrímsson et al. 2005). However, Procrustes superimposition can distribute large variances from particularly variable landmarks to the rest of the landmark configuration, a limitation known as the ''Pinocchio effect'' (Zelditch et al. 2004a, p. 119). Additionally, geometric morphometric methods are necessarily multivariate as shape is defined as a character of the entire landmark configuration (Zeldtich et al. 2004a, pp. 14–16). While multivariate methods are useful, they can be difficult to interpret (Lele and Richtsmeier 2001, p. 17). Distance data offer straightforward results that are relatively simple to interpret for both univariate and multi-

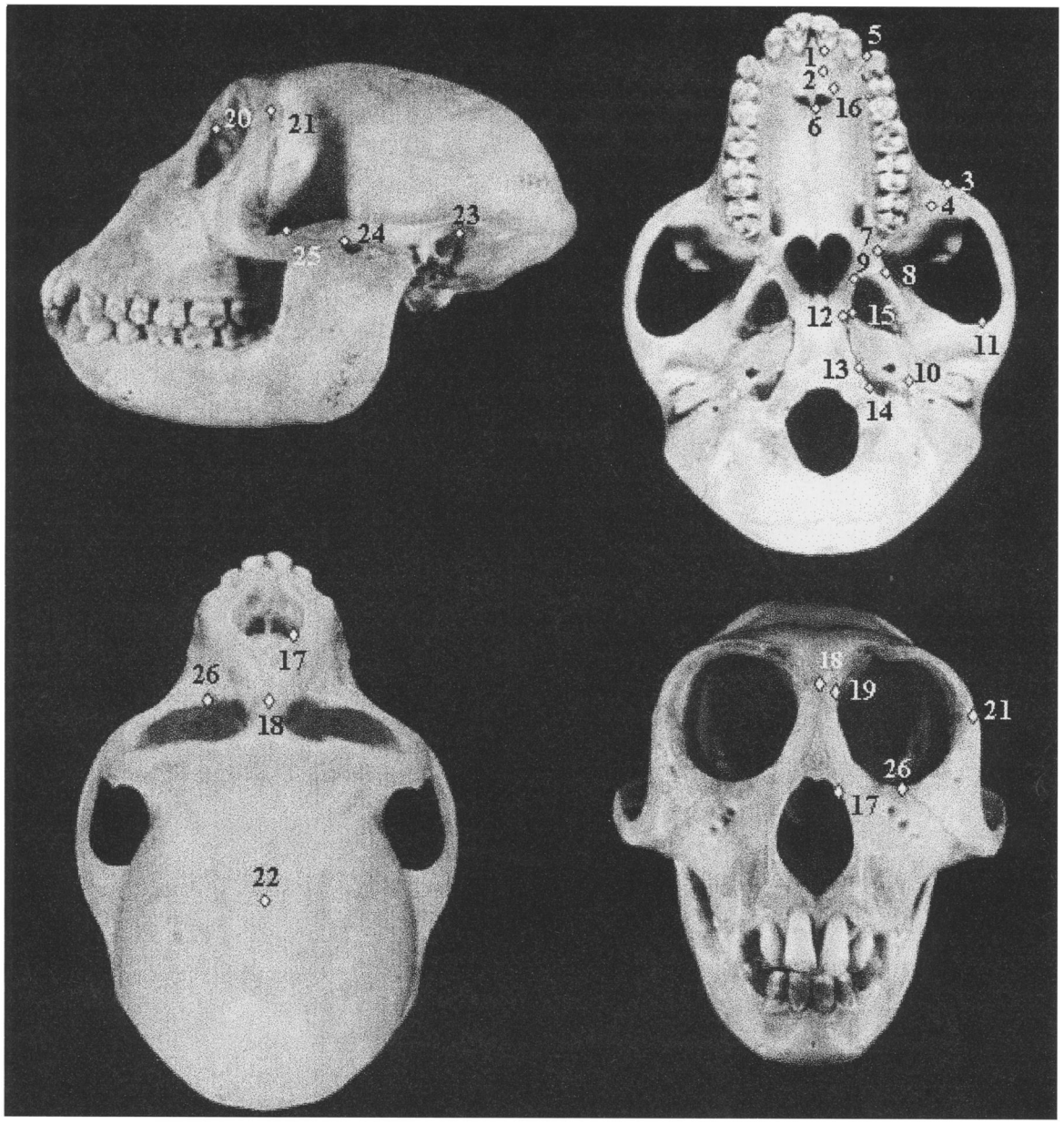

FIG. 1. Landmarks collected from macaque skulls from lateral, basicranial, superior, and frontal views. Figure shows landmarks in the midline and on the right side only; however, landmarks were digitized bilaterally.

variate analyses. However, it is difficult to visually display distance data results (Richtsmeier et al. 2002; and Zelditch et al. 2004a, pp. 2, 7), and the large number of potential variables can inflate the degrees of freedom (Zeldtich et al. 2004a, pp. 3–4).

Correlations between variance components and fluctuating asymmetry

Scatterplots of superimposed Procrustes coordinates were used to visually inspect the landmark configuration data for gross outliers using the program Morpheus (Slice 1994–1999). Such gross errors can be caused by mislabelling of landmarks or by side reversal during digitizing. Outliers were removed from the dataset and redigitized.

Further analyses for robustness of the data were performed separately for both Procrustes superimposed data and Euclidean distance data using Grubb's test (Barnett and Lewis 1994) for outliers. Outliers for both measurement error and asymmetry that were significant using Bonferroni adjustment ($P = 0.001$) were removed from further analysis. It is important to remove these data as outliers for measurement error because entry or gross measurement errors could mask FA and outliers for asymmetry due to specimen damage could artificially inflate measures of FA.

Univariate analyses

Univariate analyses were only carried out on Euclidean distance data, as analyses of landmark configurations must

TABLE 1. Interlandmark distances (see Fig. 1) used in this study that showed significant fluctuating asymmetry (FA) using Palmer and Strobeck's (2003) two-way mixed-model ANOVA. Most of the distances also showed significant directional asymmetry (DA). Distances were chosen that highlighted regions of anatomical interest (Hallgrímsson et al. 2004).

Distance	Region of skull	FA	Significant DA (*P*)
1 to 15	face	0.1980	<0.001
1 to 5	face	0.3165	<0.001
9 to 12	basicranium	0.2751	1
10 to 12	basicranium	0.5301	<0.001
10 to 21	neurocranium	0.3765	<0.001
12 to 23	basicranium	0.2630	<0.001
13 to 21	neurocranium	0.2136	<0.001
13 to 22	basicranium	0.2590	<0.001
14 to 23	basicranium	0.1514	<0.001
15 to 6	face	0.3484	<0.001
16 to 17	face	0.7006	<0.001
19 to 2	face	0.1618	<0.001
19 to 20	face	0.4052	<0.01
20 to 14	neurocranium	0.6118	1
20 to 21	neurocranium	0.5051	<0.001
21 to 11	neurocranium	0.3379	1
21 to 26	neurocranium	0.1681	<0.001
22 to 21	neurocranium	0.3969	<0.001
3 to 11	basicranium	0.4204	<0.001
3 to 16	face	0.2044	0.218
3 to 4	face	0.5548	<0.001
24 to 20	face	0.4523	<0.001
25 to 11	face	0.4079	0.581
25 to 19	face	0.2570	0.111
25 to 20	face	0.4513	<0.01
25 to 24	face	0.3527	<0.001
26 to 17	face	0.6272	<0.001
5 to 3	face	0.2804	<0.001
5 to 26	face	0.0926	0.968
5 to 7	face	0.1168	1
6 to 7	face	0.1904	0.071
7 to 17	face	0.1897	<0.001
8 to 9	basicranium	0.1922	<0.001
8 to 10	basicranium	0.3694	<0.001

be multivariate due to shape being a product of the whole configuration of landmarks (Zelditch et al. 2004a, pp. 14–16).

The univariate measure of FA was calculated using the two-way mixed-model ANOVA with sides fixed and individuals and trials as random factors, as recommended by Palmer and Strobeck (2003). We removed linear size dependence for FA by natural log-transforming all data to obtain a size-scaled measure of FA (Wright 1952; Lewontin 1966; Van Valen 1978). Specifically, we used Palmer and Strobeck's (1986, 2003) FA10 index, as this estimate of FA tests for the significance of FA after removing the effects of measurement error:

$$FA10 = 0.798[2(MS_{sj} - MS_m)/M]^{1/2}, \quad (1)$$

where MS_{sj} is the mean square for sides/individuals interaction, MS_m is the mean square for measurement error, and M is the number of replicate trials. This method of FA estimation takes the absolute difference between right and left sides, effectively truncating the distribution. The result of this truncated distribution is that the mean and standard deviation are now linked and the mean of this new distribution differs from the standard deviation of the signed asymmetry distribution by the constant 0.798 (Palmer 1994).

Distances that were insignificant for FA were eliminated from further analysis. We tested for the presence of directional asymmetry (DA) for each distance measure by an *F*-test, with $F = MS_s/MS_{sj}$, where MS_s is the mean square for sides. The signed asymmetry distributions were inspected for signs of being bimodal to check for potential antisymmetry, as it is not possible to differentiate FA from antisymmetry from the between sides variance measure (MS_{sj}; Palmer and Strobeck 2003).

The univariate phenotypic variance (V_P) was calculated as the variance of each interlandmark distance. To account for sex differences, V_P was calculated separately for each sex and then averaged. The genetic variance (V_G) was calculated from *Z*-scores standardized within sex to account for sexual dimorphism (Cheverud 1982). Because the within-sex phenotypic variances are standardized to 1.0, V_G is equal to the heritability. For this sample, the identity of the mother is known but that of the father is not. Thus, we estimated heritability using mother-offspring pairs, half-siblings, aunt-offspring pairs, and grandmother-offspring pairs following Cheverud (1982),

$$h^2 = \{[1/(k_1 + k_2)]\text{cov}[R(P_iP_i)]\}/V_P, \quad (2)$$

where k_1 and k_2 are Cotterman's *k*-coefficients, $\text{cov}[R(P_iP_i)]$ is the covariance among relatives for a phenotypic trait P_i (in this case an interlandmark distance), and V_P is the phenotypic variance.

Heritability estimates calculated from aunt-offspring and grandmother-offspring pairs were eliminated as several of the estimates were negative and made subsequent calculations of V_G and V_E impossible. Cotterman's *k*-coefficients were used as outlined by Crow and Kimura (1977). As paternity is unknown, it is not possible to determine if offspring of a given mother are half- or full-siblings, and we assumed an equal probability of being half- or full-siblings and used $k = 1/3$ as the Cotterman's *k*-coefficient for siblings. The environmental variance is obtained from the equation $V_P = V_G + V_E$ (Falconer and Mackay 1996, p. 122). This method does not account for dominance or maternal and common environment effects. Thus, it estimates broad-sense heritability, and the environmental variances are probably underestimates (Falconer and Mackay 1996).

To determine univariate relationships among variance components, linear regressions were performed for the set of significant FA variances against V_P, V_G, and V_E.

Multivariate analyses

Correlations between multivariate measures of FA, phenotypic variance, and the genetic and environmental components of variance were calculated using both Euclidean distance data and Procrustes superimposition data. We used the same methods to estimate these different multivariate components of variance for both Procrustes and distance data and therefore these methods are described only once.

To produce covariance matrices for FA, we used the difference between left and right data and averaged across the three replicate trials for each individual. The phenotypic cor-

relation matrices ($\mathbf{R}_P$) were constructed by calculating the variance/covariance matrix between traits. Matrices were constructed separately for males and females and then averaged to account for sex differences. Data used to calculate the genetic ($\mathbf{R}_G$) and environmental ($\mathbf{R}_E$) correlation matrices were *Z*-transformed within sexes to remove the effects of sexual dimorphism. $\mathbf{R}_G$ and $\mathbf{R}_E$ were calculated using the equations described by Cheverud (1982) based on mother-offspring and sibling pairs. The genetic correlation matrix was calculated as,

$$\mathbf{R}_G = [1/(k_1 + k_2)]\{\mathrm{cov}(\mathrm{R}(\mathrm{P}_i\mathrm{P}_j)]/\sqrt{\mathrm{V}_{Ai}\mathrm{V}_{Aj}}\}, \quad (3)$$

where k_1 and k_2 are Cotterman's *k*-coefficients and $\mathrm{cov}[\mathrm{R}(\mathrm{P}_i\mathrm{P}_j)]$ is the averaged cross-covariance among relatives for phenotypic values, and V_A is the additive genetic variance which is equal to h^2 as V_P is equal to 1.0. The Cotterman's *k*-coefficients used in the univariate analysis were also used to calculate the multivariate genetic correlation matrix. The $\mathbf{V}_{Ai}\mathbf{V}_{Aj}$ matrix was calculated as the cross-covariance among heritability estimates for the different traits. Estimates of h^2 were already calculated for the distance data in the univariate analysis. Heritability (h^2) estimates were also calculated for Procrustes superimposed coordinates using the same method as described for the distance data.

The environmental correlation matrix was also constructed following Cheverud's (1982) equation:

$$\mathbf{R}_E = (\mathbf{R}_P - h_i h_j \mathrm{R}_G)e_i e_j, \quad (4)$$

where $h_i = \sqrt{h_i^2}$, $e = \sqrt{1 - h_i^2}$, $\mathbf{R}_P$ is the phenotypic correlation and $\mathbf{R}_G$ is the averaged genetic correlation for mother-offspring and sibling pairs.

Matrix correlations were then calculated for comparisons between FA and $\mathbf{R}_E$, FA and $\mathbf{R}_G$, and FA and $\mathbf{R}_P$ to determine the relationships among the variance components. The statistical significance of matrix correlations for the Euclidean distance data were tested using Mantel's test (Cheverud et al. 1989). To test for the significance of the matrix correlations based on Procrustes superimposed data, we used a matrix permutation test excluding the diagonal adapted for geometric morphometrics (Klingenberg and McIntyre 1998), as implemented by the program Mace3D (Marquez 2004). This program performs a Mantel's test shuffling the data within the matrix. However, the program ensures that coordinates of a particular landmark are shuffled together. The x-, y-, and z-coordinates of a landmark are intrinsically correlated and if shuffled separately would inflate the measures of matrix correlations artificially (Klingenberg and McIntyre 1998; Klingenberg et al. 2003).

Visualization of Shape Differences

To extract the information on shape variation from the coordinate data, we used Procrustes superimposition, taking into account the inherent symmetry of the skull by using the procedure for object symmetry (Klingenberg et al. 2002). We tested for overall FA using a two-factor mixed-model ANOVA. This analysis accounts for potential asymmetry of landmarks in the median plane as well as in the paired landmarks on either side of the skull.

Procrustes shape FA was calculated as the difference of the original and the reflected and relabelled configurations for all three trials for each landmark coordinate (Klingenberg et al. 2002). For among-individual variation, we used the individual means across trials and reflection (side). To reduce the effects of sex, we calculated the mean values for each coordinate for males and females separately. We then subtracted the male means from the female means, and this difference was then added to the male data. Genotypic values were calculated for each coordinate for each individual by averaging the values of all relatives in the sample weighted by the *k*-coefficient, similar to calculations used to quantify the breeding value of an individual (Falconer and Mackay 1996, pp. 108, 114). Therefore, if an individual had a mother and two siblings in the sample, their genotypic value (GV) for a given coordinate would be calculated as

$$\begin{aligned}\mathrm{GV} = \{&(\text{value of mother} \times 0.5)\\ &+ [\text{value of sibling A} \times (0.25 + 0.5)/2]\\ &+ [\text{value of sibling B} \times (0.25 + 0.5)/2]\}/1.25.\end{aligned} \quad (5)$$

The environmental deviation was then calculated as the difference between an individual's genotypic value and its phenotypic value. Genetic and environmental covariance matrices were computed from these deviations.

To visualize the primary features of shape variation occurring due to FA, environmental and phenotypic variation, we used principal components analysis of the Procrustes data to determine shape change along each principal component. Principal component coefficients were scaled to show the extremes of variation and added to the overall shape mean, and deformations of wireframes along principal components were visualized using Morphologika (O'Higgins and Jones 1998).

Results

Univariate Analyses

Euclidean distance data was used for univariate comparisons between FA and components of variance. FA was calculated for each of the 60 distances chosen for this analysis using Palmer and Strobeck's (2003) FA10 index. For 34 of these distances there was significant FA, and 27 had significant directional asymmetry (see Table 1). Antisymmetry was not detected.

The phenotypic (V_P), genetic (V_G), and environmental (V_E) variance were also calculated for each interlandmark distance outlined in Table 1. The correlation between VE and FA derived by linear regression was relatively low ($R^2 = 0.112$) but significant ($P = 0.043$; Fig. 2A). A negative correlation of the same magnitude holds for V_G. Both correlations have an R of 0.35, indicating that approximately only 12% of the variance in asymmetry is associated with the variance in either V_E or V_G. The correlation between FA and V_P is not statistically significant (Fig. 2B).

Multivariate Analyses

Correlation matrices were constructed for FA and for the phenotypic ($\mathbf{R}_P$), genetic ($\mathbf{R}_G$), and environmental ($\mathbf{R}_E$) com-

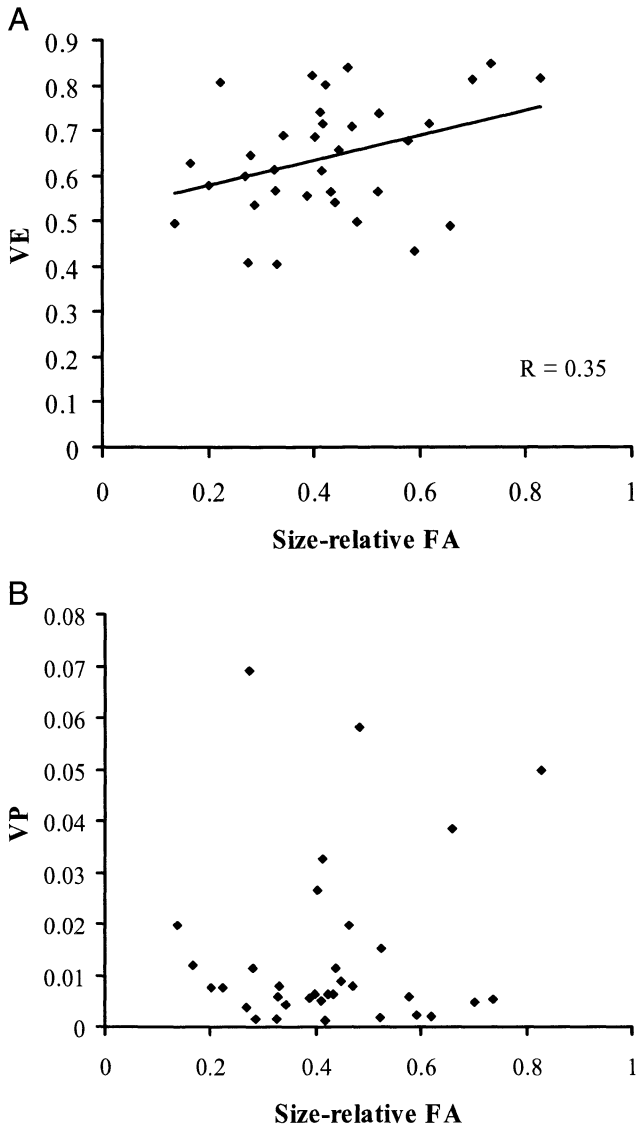

FIG. 2. Variance components plotted against size-relative fluctuating asymmetry (FA) for *Macaca mulatta* skulls. (A) Environmental variance (V_E) plotted against size-relative FA. Graph shows a positive relationship between the magnitude of FA and the magnitude of V_E. (B) Phenotypic variance (V_P) plotted against size-relative FA. Graph shows that there is no relationship between the magnitude of FA and the magnitude of V_P.

ponents of variation among individuals for the 34 interlandmark distances as well as for the superimposed Procrustes coordinates. Matrix correlations were calculated using Mantel's test for FA and $\mathbf{R}_P$, FA and $\mathbf{R}_G$, and FA and $\mathbf{R}_E$ for interlandmark distance data. All of the distance correlations were significant yet low. The correlation between FA and $\mathbf{R}_E$ is 0.164 with $P < 0.001$, between FA and $\mathbf{R}_G$ the correlation is also 0.164 with $P < 0.01$, and the correlation between FA and $\mathbf{R}_P$ is 0.185 with $P < 0.001$. Similar results were obtained using Procrustes superimposed coordinate data. The correlation between FA and $\mathbf{R}_E$ for the Procrustes data is -0.070 with $P < 0.01$, between FA and $\mathbf{R}_G$ the correlation is 0.043 with $P < 0.01$, and the correlation between FA and $\mathbf{R}_P$ for Procrustes data is 0.049 with $P < 0.01$. These results indicate that the covariance structure of asymmetry shows significant similarity with the correlation structure of phenotypic, genetic, and environmental variation.

Visualization of Shape Differences

The initial analysis of shape asymmetry using Procrustes ANOVA (Klingenberg et al. 2002) indicated that there is significant FA and directional asymmetry in the skulls (see Table 2). The presence of significant FA in these skulls allowed us to proceed with our analysis.

Figure 3 shows variation along the first two principal components for Procrustes shape variation for environmental variation, phenotypic variation and FA as deformations in three-dimensional wireframes. Visual inspection of the shape changes along each principal component illustrates that both the magnitude and direction of shape changes are similar for all three sources of variation. Variation along the first principal component shows opposite changes in anteroposterior facial length and changes in cranial vault length and height. The second principal component primarily features changes in basicranial flexion. These three-dimensional wireframe deformations allow visualization of the quantitative correlations observed between FA and V_E.

DISCUSSION

The aim of this study was to test for congruence between patterns of variation within individuals and patterns of variation among individuals using macaque skulls from Cayo Santiago. Specifically, we were interested in how the environmental component of phenotypic variation correlated with FA, as both variance components are thought to buffer development against environmental perturbations. We hypothesized that there is overlap in the developmental bases for developmental stability and canalization and predicted that traits that have high levels of environmental variation will also show high levels of FA and that the covariance structure for deviations from symmetry will be related to the covariance structure for other variance components.

Controversy surrounding the suggestion that canalization and developmental stability share common perturbations and buffering mechanisms stems from the conflicting results of

TABLE 2. Procrustes ANOVA calculated using the method for object asymmetry (Klingenberg et al. 2002). There is overall significant fluctuating asymmetry in the macaque skulls and directional asymmetry is also significant.

Source	df	SS	MS	*F*	*P*	FA10
Individual	15840	4.062	0.000256			
Reflection	71	0.037	0.000521	24.20	<0.001	
Individual × reflection	15620	0.336	0.000022	593.44	<0.001	0.002136
Measurement error	63206	0.002	0.000000			

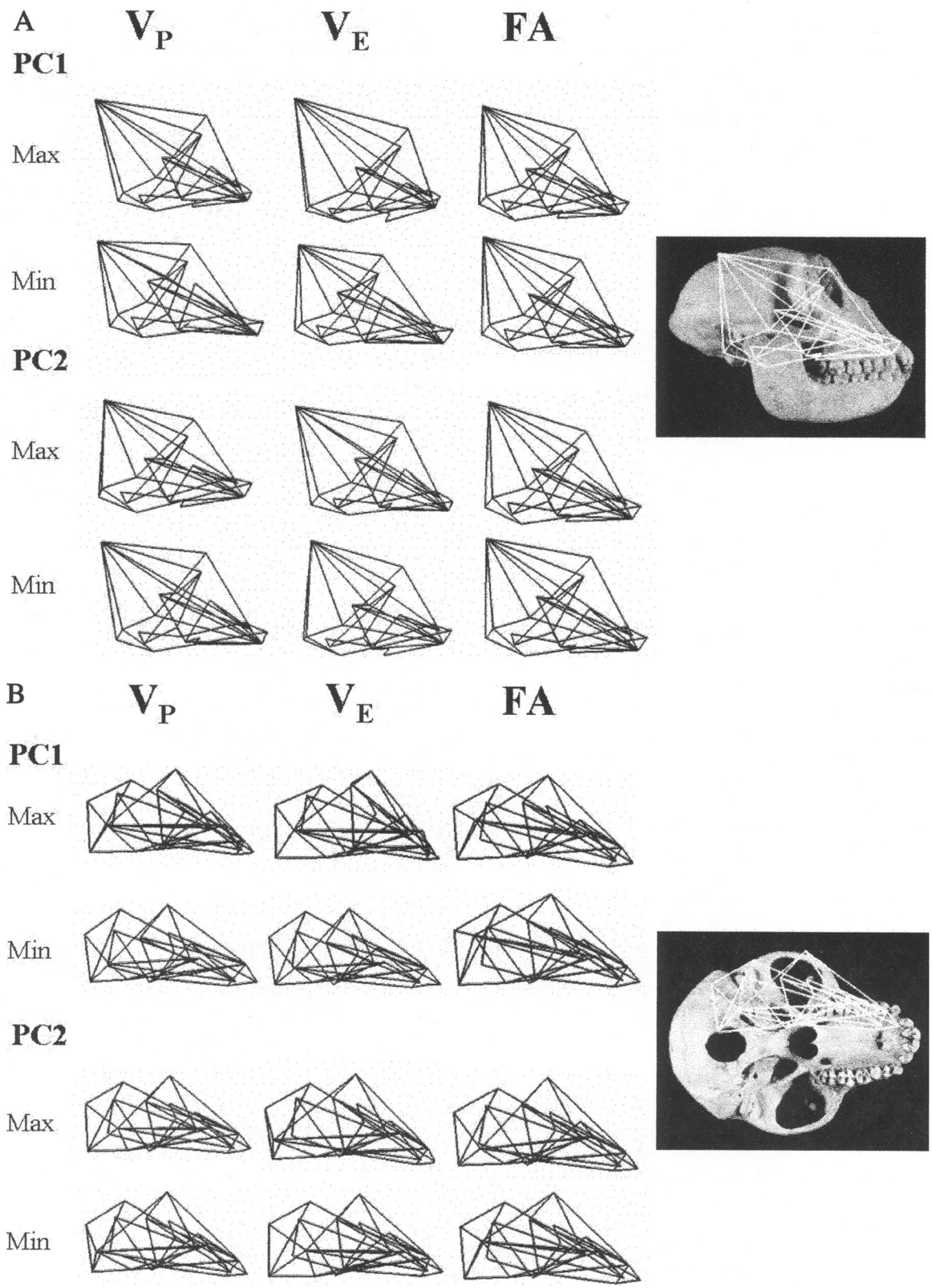

FIG. 3. Variation along the first two principal components in lateral (A) and basicranial (B) views. The wireframe linking the points used in the analysis was arbitrarily constructed to aid visualization of both the magnitude and direction of variation within individuals (fluctuating asymmetry, FA), phenotypic variation (V_P), and the environmental component of the phenotypic variation (V_E). For each principal component, a wireframe is shown for the upper and lower extremes of variation along that component in the sample.

previous empirical studies. Several studies have found significant associations between FA and either phenotypic or environmental variances for a variety of model organisms. In mice, congruence between among- and within-individual variance was found for both skeletal limb elements (Hallgrímsson et al. 2002) as well as for mandibular traits (Klingenberg et al. 2003). Likewise, significant correlations between FA and among-individual variation have been found for wing traits of *Drosophila* (Klingenberg and Zaklan 2000), tsetse flies (Klingenberg and McIntyre 1998), and bumblebees (Klingenberg et al. 2001). Other invertebrate traits have also demonstrated an association between FA and phenotypic variance (Clarke 1998) and FA and environmental variance (Woods et al. 1999). This relationship has even been found in plants. Queitsch et al. (2002) found that both developmental stability and canalization were reduced in the plant *Arabidopsis thaliana* when Hsp90 was compromised. The results of these studies would indicate that the mechanisms involved in buffering perturbations within individuals are the same as the mechanisms used to buffer perturbations among individuals, suggesting that the question posed by this study has been answered and the results redundant.

However, other studies using similar model systems have found no correlation between FA and among-individual variance. Debat et al. (2000) found no correlation between FA and phenotypic variation in a study of the skulls of two inbred house mouse populations and their F_1 hybrids. This lack of congruence was also noted by Réale and Roff (2003) for limb traits of the cricket *Gryllus firmus*. Particularly convincing are the studies involving Hsp90 in *Drosophila* (Rutherford and Lindquist 1998; Rutherford 2000) as they offer a specific mechanism for canalization. Rutherford and Lindquist (1998) found that compromising Hsp90 in *Drosophila* either by pharmacological treatment or by mutation led to increased variance in several traits, and this variance persisted in offspring even in individuals where Hsp90 function had been restored. Rutherford (2000) then tested the potential role of Hsp90 as a mechanism for developmental stability in *Drosophila* by measuring FA in mutant and wild-type flies. She found that FA did not increase in mutants and concluded that developmental stability and canalization have separate underlying processes.

The results of the present study fall somewhere between complete congruence and no congruence between FA and environmental variance. Our univariate analysis of interlandmark distances shows a low but significant correlation between FA and V_E. That is, traits exhibiting higher levels of asymmetry deviations also exhibit greater levels of environmental variance. We suspect that our univariate correlations are underestimated due to sampling and measurement error. Even so, there is still a great deal of variance that is not explained by congruence between FA and V_E. Likewise, multivariate analyses of both interlandmark distance data and Procrustes data show this same low but significant pattern of correlation. These multivariate results indicate that covariances of absolute symmetry deviations partly correspond to covariances of mean deviations at the individual level. Although these associations are weak, both results suggest the existence of some degree of overlap between the developmental bases for developmental stability and canalization.

Our results are based on analyses of both Procrustes superimposed landmark data as well analyses of interlandmark distances. It is important to recognize that these approaches capture different aspects of variation. In the Procrustes dataset, landmark values have been normalized for centroid size. For this reason, covariance results from coordinated departures from a mean shape. In such a dataset, for instance, isometric covariation among structures is cancelled out by the Procrustes superimposition process. In interlandmark distance data, however, isometric and allometric relationships among structures contribute to covariance. Similar results were obtained using both approaches. However, the fact that lower matrix correlations were obtained from the Procrustes data suggests that removing the isometric component of covariance from the data weakens the relationship between FA and environmental variance.

Zelditch et al. (2004b) pointed out that variance results from a balance between perturbations or mechanisms that create variance and mechanisms that reduce or limit variance. Therefore, congruence between variance components, such as FA and V_E, may arise if the developmental causes of variance or the mechanisms that reduce variation are similar, or if there are overlapping elements for both the causes and buffering mechanisms. Partial congruence may likewise occur between the components of variance if there is only partial overlap of the elements responsible for creating and removing variance during development. We interpret our results using this multifactorial view of variance as well as Siegal and Bergman's (2002) view that canalization is an emergent property of development. Therefore, we interpret our results as indicating that there is some overlap in either the perturbations creating the variance, the mechanisms used to buffer perturbations, or that there is partial overlap of both the perturbations and the buffering mechanisms for both within- and among-individual variance. We also suggest that these perturbations and buffering mechanisms are generic properties of development and therefore doubt the need for specific canalizing or developmental stability genes.

Our data do not allow us to determine the specific developmental processes that created the partial congruence of variance patterns in this macaque population. However, we speculate on some potential generic properties of development that could account for varying degrees of correlation between FA and among-individual variance. These potential developmental processes are divided hierarchically. We have divided these mechanisms into these different levels to illustrate that emergent properties of development are acting at all organismal levels, but we stress that several of these processes from each of the three levels may be responsible for resulting patterns of variance. This discussion does not provide an exhaustive exploration of the potential mechanisms involved; it is only meant to demonstrate that generic properties of the developing organism can explain a multitude of patterns of within- and among-individual variance.

Mechanical Influences on Bone Growth

The functional matrix hypothesis as described by Moss (1968) is based on the premise that the skull carries out specific functions and posits that growth of skeletal units,

once initially formed, is a function of the soft tissues and functional spaces in which the bony elements are embedded. He describes two types of functional matrices, a periosteal type and a capsular variety. Periosteal functional matrices act on skeletal elements directly through soft tissues, particularly through muscular action. Capsular functional matrices such as the pharyngeal cavity, orbits, and the brain act indirectly on skull growth by spatially translating skeletal units (Moss 1968).

Variance in these functional matrices could potentially create variance in skull shape. Muscular action on cranial elements is a probable source of both within- and among-individual variance. In a series of papers, Herring and colleagues (Herring and Teng 2000; Rafferty et al. 2000; Herring et al. 2002) have reported that muscle contraction in juvenile pigs increases strain in the braincase. This increased strain is localized to specific portions of the skull that correspond directly to the muscular action (Rafferty et al. 2000). They found that contraction of the temporalis muscle increased strain in the parietal bone and the interparietal suture, whereas masseter contraction acted to increase strain in the frontal bone and interfrontal suture (Herring and Teng 2000). Given the relatively large size of the muscles of mastication in macaques, it is reasonable to assume that these muscles will play some role in the resulting variation found in macaque skulls. Wood and Lieberman (2001) argued that skeletal traits associated with relatively large strains are more variable than skeletal elements subjected to weaker strains. Therefore, individuals with larger muscles of mastication would be expected to have increased variance in those skeletal elements directly affected by muscular contraction such as the parietal and frontal bones. Likewise, individuals with asymmetrical muscular development as a result of either chewing side preference or simply a product of initial development of the muscles are expected to have increased FA in those elements directly affected by the muscles of mastication.

However, Daegling (2004) argued that not all skeletal elements will respond similarly to increased strain. He used the mandible as an example, suggesting that while the mandibular condyle and mandibular coronoid process may be subjected to similarly high strains, the affect on condylar variance is expected to be less than that of the coronoid. This expectation is based on the relatively greater importance of precise development of the condyle, as it is involved in the temporomandibular joint, whereas the coronoid functions merely as a site of muscle attachment. Daegling did not propose a developmental mechanism by which lower variances for functionally more important traits would be achieved.

Zelditch et al. (2004b) offered another explanation for variable effects of muscular contraction on skeletal elements. They found that variance decreases with developmental time in both the cotton rat and house mouse skull. Increased canalization of skull shape with time was suggested to result from the maturation and greater organization of neuromuscular control that develops throughout ontogeny (Zelditch et al. 2004b). This predicts that skeletal elements that form early in development show greater degrees of variance because they are subjected to less organized neuromuscular control for a greater proportion of their growth and development. This hypothesis can be explored through future studies as well as experimental models.

It is widely accepted that brain growth and development plays a major role in early cranial development, particularly in primates. Brain growth and development seems to have a greater affect on the development of the neurocranium than on either the basicranium or facial skeleton. However, the compressive forces exerted by the brain on the basicranium are thought to cause resorption and create the superiorly oriented concavities of the cranial base (Herring 1993). Lieberman et al. (2000) suggested that the retrognathic orientation of the human facial skeleton is a result of increased brain size in humans compared with other primates. Brain growth is thought to apply quasi-static, equibiaxial tensile strain to the neurocranium, and this strain is thought to stimulate sutural growth (Henderson et al. 2004). It is argued that this tension from the brain is necessary for normal neurocranial development, as individuals with anencephaly exhibit highly abnormal braincase development (Herring 1993). We expect that the equibiaxial strain produced by the expanding brain will have a relatively global effect on the neurocranium in contrast to the localized effects of muscular contraction.

Architecture of Developmental Systems

Three generic properties of developmental systems have been suggested to influence variability: nonlinear dynamics, thresholds, and redundancy (Emlen et al. 1993; Klingenberg and Nijhout 1999; Klingenberg 2003; Hallgrímsson et al. 2004). Nonlinear relationships between developmental parameters and phenotypic outcomes can potentially explain the often seemingly nonsensical effects of mutations or environmental perturbations on disparate developmental products. Klingenberg and Nijhout (1999) constructed a model that demonstrated a nonlinear relationship whereby they found genetic variation in FA at the phenotypic level, despite constant developmental noise at the genotypic level. Potential empirical evidence for this relationship is offered by Leamy et al. (2002), who found that there is an epistatic genetic basis for FA in the mouse mandible. Thresholds can likewise alter expected developmental outcomes. Depending on the relationship between the developmental determinant and the phenotypic outcome and where along that curve an individual lies, thresholds can either dampen or amplify variation of developmental products. Redundancy is another property of developmental systems that modulates variation from the genotype to the phenotype. Redundancy and complexity of developmental components can influence variability. Lande (1977) pointed out that a process composed of a large number of independently varying components will have a lower variance than a process comprised by fewer components, assuming that the variances of the components remain the same. Redundancy, which is commonly seen in the overlapping functions of developmental genes (Carrol et al. 2005), increases the number of components influencing a developmental process. Redundancy in genetic networks regulating development also buffers development against genetic perturbations that disrupt the function of specific components.

All three of these properties of developmental architecture can be effective in reducing variation at the phenotypic level

by essentially hiding the effects of variation at the cellular and molecular level. These properties can also amplify and distribute variation at the cellular and molecular level in unpredictable ways at the phenotypic level.

Developmental Processes

Cranial bones are derived from two cellular primordia: the cranial neural crest and paraxial mesoderm (Noden 1978). Processes that transform these primordia into cranial elements are complex, and this complexity can influence the degree of phenotypic variance in the final skeletal product. If we focus on the neural crest only, we can readily see the extent of these complexities.

A series of coordinated events must occur to ensure that neural crest cells develop into proper facial primordia including: induction, differentiation, and migration. Neural crest induction is a two-step process involving first the dorsal mesodermal induction of neural ectoderm, and second the epidermal ectodermal induction of neural crest at the epidermal/neural ectodermal border (Hall 1999). Many of the factors that are known to be involved in neural crest induction are involved in a variety of developmental pathways. Additionally, many of these factors have both inducing and inhibitory control of neural crest. For example, *Dorsalin-1*, a member of the TGF-β superfamily, is capable of both inducing and inhibiting neural crest (Hall 1999). Neural crest must undergo a process of differentiation to properly form cranial elements. Differentiation is dependent on a variety of transcription factors, the expression or lack of expression of Hox genes as well as the region of origin in the hindbrain (Francis-West et al. 2003; Gilbert 2003). Many of the transcription factors required for differentiation are also necessary for induction and migration adding yet another level of interaction and complexity.

The proper migration of cranial neural crest cells is crucial for normal craniofacial development. To facilitate migration, it is important that there is a cell-free pathway within the extracellular matrix (Hall 1999). Neural crest cells produce different proteases such as plasminogen activators (Hall 1999) and ADAM-13 (Francis-West et al. 2003) to degrade the extracellular matrix, making a pathway for migration. While it is necessary to clear away the extracellular matrix to some degree, the matrix is rich in glycosaminoglycans such as fibronectin, which help to control migration by inhibiting or promoting cell-to-cell and cell-to-matrix adhesion (Hall 1999).

The large number of processes and factors involved in neural crest induction, differentiation, and migration offer a mechanism of buffering any errors that may arise within a single process or with an individual factor. However, the interdependence of these processes and factors can also amplify such errors. Therefore, the same processes may be involved in both producing and reducing variance at the cellular level. Likewise, it is possible that errors may be localized or they may have global effects, creating either within-individual or among-individual variance, respectively. The above example, albeit incomplete, of the control of neural crest cells demonstrates that processes of complex development, such as that of the mammalian skull, are sufficient to both create and reduce variance.

Variation within the skull in the developmental processes that influence cranial development would contribute to concordance between FA and among-individual variation if the relevant developmental processes vary in their sensitivity to perturbation among individuals. One such process would be ossification. Passing through cartilaginous precursors, the chondrocranium is exposed to a different sequence of developmental events than the dermatocranium or viscerocranium (Hall and Miyake 2000). If the developmental architecture of these different processes vary such that they respond differently to perturbation and those responses are similar at the within- and among-individual levels, then ossification pattern would generate concordance between developmental stability and canalization across traits. There are other processes for which similar arguments could be made. Hypotheses of this kind can be explored by future studies that combine natural patterns such as those studied here with the effects of mutations that affect specific aspects of craniofacial development.

Conclusion

We argue that generic processes of development are sufficient for both the production and reduction of phenotypic variance. Moreover, we suggest that these processes work in concert and that the balance between processes that introduce variance and ones that help remove variance determines the degree of phenotypic variance. This mechanistic view of the determinants of variability can explain any degree of congruence between FA and among-individual variance. The results of the present study show only partial congruence, which may be explained by the interplay of processes that act locally, potentially inflating or reducing FA, and more global processes that may increase or decrease among-individual variance. We outline some potential examples of developmental processes acting within the mammalian skull that may be responsible for both within- and among-individual variance and the relationship between the two.

In the present study, we did not test specific mechanisms modulating variance within and among individuals. Studies designed to determine the effects of specific yet generic developmental processes on among- and within-individual variation are necessary to understand the underlying mechanisms of developmental stability and canalization. Therefore, we conclude that our results of partial congruence between FA and environmental variation provide support for our hypothesis that overlapping processes regulate developmental stability and canalization. However, we acknowledge that a more mechanistic approach to this question is required to fully substantiate this conclusion. We also suggest that future mechanistic approaches focus on the emergent properties of developmental genetic architecture that may modulate variability rather than searching for potential processes that are specific to either developmental stability or canalization.

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