Evolution of Morphological Integration. I. Functional Units Channel Stress-Induced Variation in Shrew Mandibles

Alexander V. Badyaev[1,*] and Kerry R. Foresman[2]

1. Department of Ecology and Evolutionary Biology, University of Arizona, Tucson, Arizona 85721;
2. Division of Biological Sciences, University of Montana, Missoula, Montana 59812-1002

abstract: Stress-induced deviations from normal development are often assumed to be random, yet their accumulation and expression can be influenced by patterns of morphological integration within an organism. We studied within-individual developmental variation (fluctuating asymmetry) in the mandible of four shrew species raised under normal and extreme environments. Patterns of among-individual variation and fluctuating asymmetry were strongly concordant in traits that were involved in the attachment of the same muscles (i.e., functionally integrated traits), and fluctuating asymmetry was closely integrated among these traits, implying direct developmental interactions among traits involved in the same function. Stress-induced variation was largely confined to the directions delimited by functionally integrated groups of traits in the pattern that was concordant with species divergence—species differed most in the same traits that were most sensitive to stress within each species. These results reveal a strong effect of functional complexes on directing and incorporating stress-induced variation during development and might explain the historical persistence of sets of traits involved in the same function in shrew jaws despite their high sensitivity to environmental variation.

Keywords: developmental plasticity, morphological integration, modularity, *Sorex* shrews, stress, variation.

Evolutionary change at the level of phenotype is a result of both individual developmental processes that produce variation and the population dynamics of natural selection that sorts this variation (e.g., Schmalhausen 1949; West-

* Corresponding author; e-mail: abadyaev@email.arizona.edu.

Eberhard 2003). Interdependency of these processes is widely recognized—an outcome of selection for the internal cohesiveness of an organism during development determines the range of phenotypes that will be subject to external selection (Whyte 1965; Schlichting and Pigliucci 1998; Fusco 2001; Arthur 2002).

However, the relative importance of internal and external selection in determining evolutionary change is a debated issue (Arthur 2001; Chipman 2001; Dworkin et al. 2001). Theoretical and empirical studies point to a primacy of developmental processes in directing evolutionary change. It is argued that the complexity of developmental pathways and networks can lead to environmental and genetic canalization with little environmental input and thus limit the range of phenotypes available to current natural selection (Baatz and Wagner 1997; Waxman and Peck 1998; Meiklejohn and Hartl 2002; Salazar-Ciudad and Jernvall 2002; Siegal and Bergman 2002). Indeed, some aspects of early ontogeny seem to be remarkably canalized against external environments (e.g., Helm and German 1996; Arthur and Farrow 1999; Hallgrimsson 1999). There are also examples of primary importance of environment-invariant developmental mechanisms late in ontogeny; for example, in the cotton rat (*Sigmodon fulviventer*), the integration of a foraging apparatus that is required for chewing develops before the actual transition to chewing from weaning (Zelditch et al. 1992). Similarly, despite a variety of sex-determining mechanisms and the rapid evolution of developmental processes that produce alternative phenotypes within each sex, no intermediate forms between sexes or between alternative phenotypes within each sex are produced, even in the absence of external selection against such intermediates (Emlen and Nijhout 2000; Zarkower 2001; Badyaev 2002).

Other studies reveal the transitory nature of developmental constraints, suggesting that external natural selection is a dominant force in shaping phenotypic diversity. For example, Beldade et al. (2002) showed that strong natural selection can overrule close developmental integration of traits and result in novel developmental pro-

cesses (see also Brakefield 2003; Muller 2003). Similarly, in many animals, sexual dimorphism in size that is favored by external selection is accomplished despite the shared developmental processes between the sexes (Badyaev 2002).

The degree to which environmental pressures are incorporated into an organism's ontogeny depends on their historical recurrence and the ability of existing developmental processes to accommodate changes favored by these pressures (Lively 1986; West-Eberhard 1989; Jablonka and Lamb 1995; Simons 2002; Emlen et al. 2003; Badyaev 2004). Thus, differences among organismal traits in response to the current environment may reflect different histories of past selection. For example, it might be beneficial for an organism not to modify its development under pressures of the current environment if this environment is rare. However, the strength and direction of natural selection differ between parts of a phenotype. Some traits (such as foraging apparatus) may experience recurrent and fluctuating directional selection that favors rapid transformations in response to changing environments, whereas other parts of a phenotype might be under concurrent stabilizing selection favoring environmental canalization (Olson and Miller 1958; Wagner 2001). A combination of a long-term stabilizing selection on the entire organism with strong and fluctuating directional selection on a few organismal components favors the evolution of modular organization where adaptive modifications of modules of characters can be accomplished with minimum interference with other parts of the phenotype (Simpson 1953; Berg 1960; Wagner and Altenberg 1996; Kirschner and Gerhart 1998).

The study of patterns of variation in fluctuating asymmetry—random accumulation of small developmental errors in bilateral traits on the left and right sides of the body—provides an opportunity to examine constraining and interacting effects of complex developmental processes and thus the relative importance of internal and external selection (Nijhout and Emlen 1998; Emlen et al. 2003; Klingenberg 2003). Traits become developmentally integrated when they originate from a common precursor, share common developmental pathways, partition the same resource during growth, or have coordinated development maintained by organism-wide signaling (Riska 1986, 1989; Klingenberg and Nijhout 1998; Stern and Emlen 1999; Badyaev and Young 2004). Covariation of signed fluctuating asymmetries is expected when traits share direct developmental links, and thus it should be stronger within a set of developmentally integrated traits (Klingenberg 2003 [also see for review of exceptions]). Moreover, a comparison of patterns of among-individual variation and fluctuating asymmetry reveals similarity in developmental processes that produce variation in trait size and

those that regulate a trait's environmental canalization. Comparison of these patterns across species allows inferences about similarity in genetic and environmental canalization of trait variation as well as historical persistence of developmental complexes.

Here we investigate patterns of stress-induced individual variation in developmental errors (a fluctuating asymmetry) in bilaterally symmetrical lower mandibles of four closely related species of soricid shrews (*Sorex monticolus*, *Sorex vagrans*, *Sorex cinereus*, and *Sorex hoyi*). Several aspects of the biology of these species make them uniquely suited for our study. First, ossification of the shrew skull and lower jaw coincides with the onset of functional use (i.e., postweaning independent foraging; Foresman 1994). Consequently, patterns of morphological integration in shrew mandibles (units corresponding to muscle attachment points; Badyaev and Foresman 2000) might be shaped by current functional demands. Second, the short generation time of shrews (12–18 mo) in relation to annual seasonal variation, their limited dispersal, nearly continuous foraging, small size (the species in this study are the smallest mammals in the world, e.g., *S. hoyi*, 2 g), and rapid growth might favor their high sensitivity to local environmental variation during development (Zakharov et al. 1991; Pankakoski et al. 1992; Badyaev et al. 2000).

We first show that in four species of shrews, patterns of within-individual developmental variation induced by stress are influenced by existing functional structures. Second, we show that the patterns of morphological integration are similar between the species, implying a long historical persistence of developmental processes that produce functional integration. We then document that species differ in the same traits that are the most sensitive to stress within each species. We discuss the evolutionary importance of this nonrandom channeling of developmental variation and the apparent concordance between genetic and environmental canalization in shrew mandibles.

Material and Methods

Data Collection

Field protocols and techniques are described elsewhere (Badyaev et al. 2000), thus here we focus on details most pertinent to this study. We carried out this study in June–August 1993–1999 on eight experimental sites (6–28 ha) located within 32 km of one another in the Swan River Valley of western Montana. Each study site contained control plots (untreated at the time of sampling) and plots where the entire forest overstory was removed by Plum Creek Timber Company during the summer before the sampling. Overstory removal drastically changed site mi-
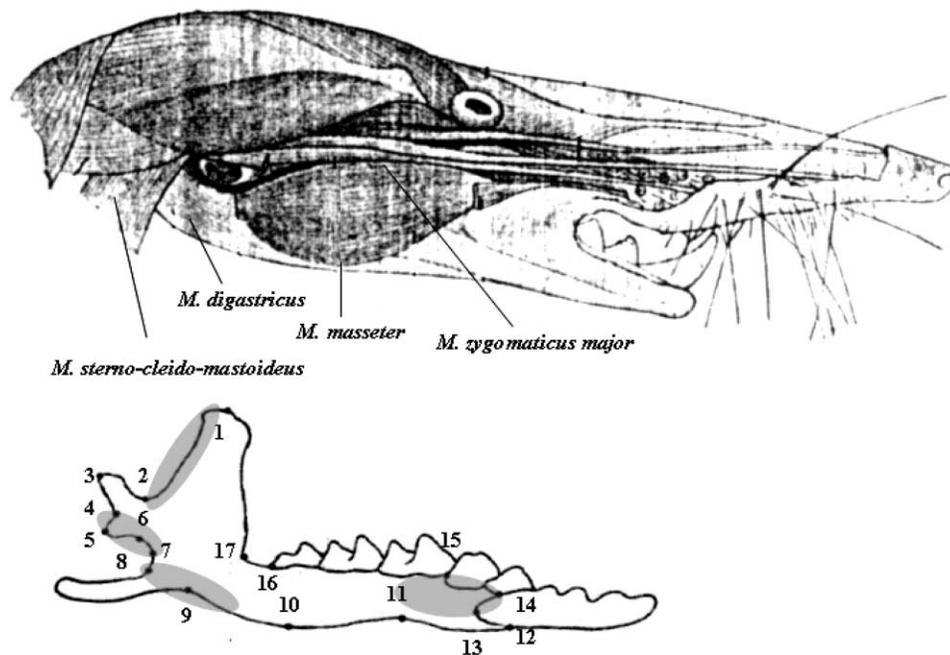
Figure 1: *Sorex* shrew skull and outline of the lower mandible. Shaded areas indicate units that are functionally integrated through attachment of the same muscle, and numbers show 17 landmarks homologous among four species.

croclimate and altered abundance and composition of arthropods, which are the main diet of shrews (Badyaev et al. 2000). All four locally coexisting shrew species (*Sorex monticolus*, *Sorex vagrans*, *Sorex cinereus*, and *Sorex hoyi*) were trapped with a grid of pitfall traps or Sherman live traps. We aged captured shrews based on tooth wear (Dannelid 1994) and restricted our analyses to 2–3-mo-old shrews that were born in May–June of the year of sampling. Shrews were weighed to the nearest 0.01 g on an electronic balance and sexed by dissection.

We had complete data on 726 individuals of four species: *S. monticolus* (control: 52 males and 50 females; vegetation removal [VR hereafter]: 45 males and 41 female); *S. vagrans* (control: 53 males and 64 females; VR: 48 males and 59 females); *S. cinereus* (control: 46 males and 43 females; VR: 74 males and 44 females); and *S. hoyi* (control: 28 males and 34 females; VR: 17 males and 28 females). The skulls were cleaned in a dermestid beetle colony, and the left and right sides of lower mandible were separated. We then photographed images of the left and right side of each mandible under 7.5 × magnification using an Olympic SZH stereo photomicroscope. The analyses of digital images were conducted with Mocha 1.2 and with SigmaScan 5.0 Pro software (Jandel Scientific 1994–2000). In each of the two replicas, specimens were repositioned, photographed, and measured (see Badyaev and Foresman 2000 for details). For some of the analyses, sex-related

variation was removed from the data in general linear models, and standardized residuals were used.

To describe functional integration in the shrew mandible, we selected landmarks associated with muscle attachment points and functionally related units of dentition (Ärnbäck-Christie-Linde 1907; Kindahl 1959; Dötsch 1982; shaded areas in fig. 1). We identified landmarks as "integrated" when they were associated with an attachment of the same muscle. Graphic representations of attachment areas of *Musculus masseter*, *Musculus digastricus*, and *Musculus sterno-cleido-mastoideus* were expanded compared with previously published scheme (Badyaev and Foresman 2000) to provide more conservative estimate due to standardization of mandible shape across a larger range of species sizes than those considered in the previous study.

Data Analysis

Variation in mandible size was eliminated before the analyses by scaling all specimens to unit centroid size. Left mandibles were then reflected to their mirror images by assigning a negative sign to their *x* coordinates. We applied a single generalized orthogonal least-squares fit (Procrustes superimposition; Rohlf and Slice 1990) to align simultaneously the landmark configurations from four species, two treatments, 726 individuals, two body sides, and two replicas (after Klingenberg and McIntyre 1998; Badyaev

and Foresman 2000). Variance in the set of aligned landmark configurations (hereafter Procrustes coordinates) was then partitioned in ANOVA models (Goodall 1991). The individual and treatment were entered as a random effect, body side was entered as a fixed effect, and the individuals were nested within treatments.

To partition the effects of each landmark on overall variation in mandible shape, we first summed x and y mean squares (MS) of each landmark and computed variance components of MS according to the expected MS for each of the effects (Klingenberg and McIntyre 1998). We analyzed the covariance matrices of the Procrustes coordinates on the basis of the expected MS-computed matrices of sums of squares and cross-products for the among-species, between-treatment, among-individual, between-sides, and between-replicas variation (after Klingenberg and McIntyre 1998). To describe patterns of covariation in the landmarks due to each effect, we derived principal components (PC) of each of the matrices as similar displacement of landmarks from their consensus position. To examine similarity between among-species, between-treatment, among-individual, and within-individual patterns of landmark displacement, we computed the angles between the first PC as $\alpha = \arccos\,[a'b/(a'ab'b)^{0.5}]$, where a and b are the eigenvectors to be compared. Statistical significance and distribution of vector angles were obtained with resampling of the within-sample PC coefficients for each effect.

Results

Integration within Functional Complexes

In each species, landmarks in functional complexes showed strongly coordinated displacement due to individual variation and fluctuating asymmetry (fig. 2; figs. A1–A3 in the online edition of the *American Naturalist*). The landmark displacement was concordant with a priori designation of functional units based on muscle attachment points (fig. 1). Under the control condition, individuals were most similar in landmark displacement in *Sorex hoyi* (PC1 accounted for 51% variation in landmark displacement), followed by *Sorex vagrans*, *Sorex monticolus* (33.9% and 33.8%), and *Sorex cinereus* (23.9%; fig. 2; figs. A1–A3). Landmark displacement was also similar among individuals raised under stressful conditions. In *S. monticolus* and *S. cinereus*, individuals were more similar in landmark displacement under stressful conditions (PC1 accounted for 46.6% and 27.2%) compared with control conditions. Under both treatments, patterns of displacement were most similar for functionally integrated landmarks (vector correlations [r_v] between patterns of displacement in two treatments: *S. monticolus*: $r_v = 0.91 \pm 0.15$ SD; *S. vagrans*: $r_v = 0.93 \pm 0.15$; *S. cinereus*: $r_v =$

0.82 ± 0.21; *S. hoyi*: $r_v = 0.58 \pm 0.19$; fig. 3). Patterns of displacement of nonintegrated landmarks were distinct among individuals in *S. cinereus* (nonintegrated landmarks: $r_v = 0.42 \pm 0.20$) and in *S. hoyi* (nonintegrated landmarks: $r_v = 0.14 \pm 0.21$; fig. 3) but were similar in *S. monticolus* (nonintegrated: 0.77 ± 0.17) and *S. vagrans* (nonintegrated: 0.82 ± 0.19; fig. 3).

Fluctuating asymmetry was closely integrated throughout the mandible and patterns of landmark displacement due to fluctuating asymmetry reflected functional integration (fig. 2; figs. A1–A3). Under the control condition, integration of fluctuating asymmetries was high in all species. Principal component 1 accounted for 50.1% of within-mandible landmark displacement in *S. monticolus*, 40.2% in *S. vagrans*, 33.4% in *S. cinereus*, and 48.7%. in *S. hoyi*. In all species, integration of fluctuating asymmetries throughout the mandible was lower under stressful conditions. Landmark displacement was similar between control and stressful treatments, especially for the landmarks that were parts of functional complexes (r_v between patterns of displacement in two treatments: *S. monticolus*: integrated 0.69 ± 0.18, nonintegrated 0.34 ± 0.10; *S. vagrans*: integrated 0.88 ± 0.19, nonintegrated 0.69 ± 0.09; *S. cinereus*: integrated 0.84 ± 0.17, nonintegrated 0.70 ± 0.21; *S. hoyi*: integrated 0.79 ± 0.24; fig. 3). Displacement of nonintegrated landmarks due to fluctuating asymmetry was distinct between the two treatments in *S. hoyi* (nonintegrated $r_v = 0.34 \pm 0.27$).

Covariation of Among-Individual Variation and Fluctuating Asymmetry

In all species in functionally integrated landmarks, landmark displacement due to among-individual variation was similar to the displacement due to fluctuating asymmetry (fig. 2; figs. A1–A3). Vector correlations of among-individual variation with fluctuating asymmetry for integrated landmarks were as follows: for *S. monticolus*, control: 0.82 ± 0.11, stress: 0.66 ± 0.09; for *S. vagrans*, control: 0.89 ± 0.07, stress: 0.88 ± 0.28; for *S. cinereus*, control: 0.60 ± 0.06, stress: 0.88 ± 0.19; for *S. hoyi*, control: 0.74 ± 0.15, stress: 0.85 ± 0.22 (fig. 4). In nonintegrated landmarks, the vector correlations of among-individual and fluctuating asymmetry displacements were distinct in both treatments for *S. cinereus* (control: 0.34 ± 0.18; stress: 0.21 ± 0.18) and *S. hoyi* (control: 0.42 ± 0.21; stress: 0.19 ± 0.18) and under stressful conditions in *S. monticolus* (control: 0.72 ± 0.08; stress: 0.24 ± 0.09; $t = 3.40$, $P < .05$). In *S. vagrans*, displacement due to among-individual and fluctuating asymmetry was similar in integrated and nonintegrated landmarks in both treatments (control: 0.80 ± 0.15; stress: 0.64 ± 0.16).
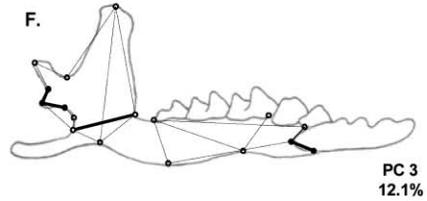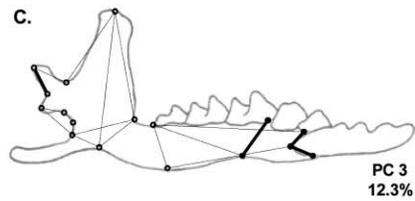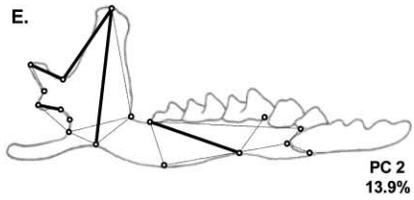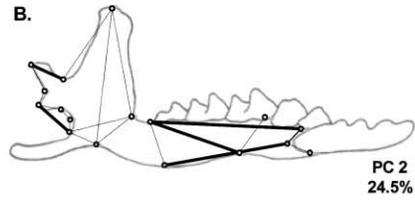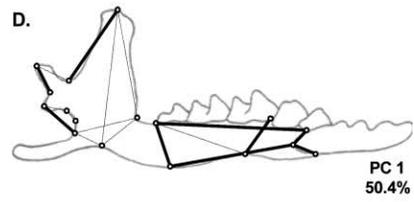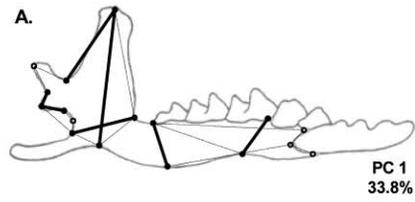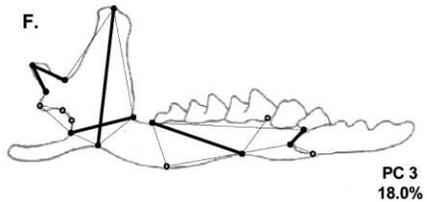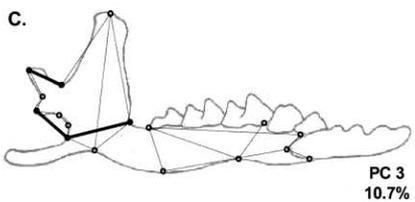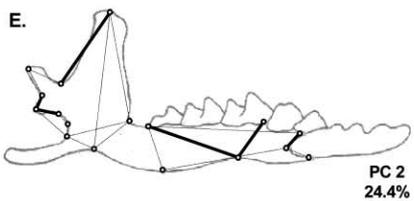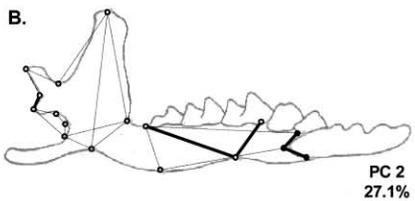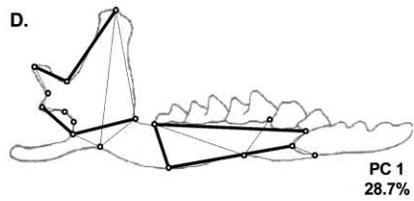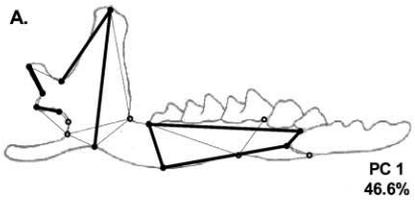
Figure 2: Integration in *Sorex monticolus* mandible shown as concordant displacement of landmarks due to among-individual variation (*I, A–C; II, A–C*) and fluctuating asymmetry (*I, D–F; II, D–F*) control (*I*) and vegetation removal (*II*) treatments. Jointly displaced landmarks (that share PC coefficients more than 0.24 [isometric loading with $n = 17$ landmarks]) are shown with thick lines. Numbers show percent of variation in the corresponding Procrustes mean squares of among-individual variation (*left column*) and within-individual variation (fluctuating asymmetry; *right column*), accounted by a corresponding PC. See figures A1–A3 in the online edition of the *American Naturalist* for *Sorex vagrans, Sorex cinereus,* and *Sorex hoyi.*
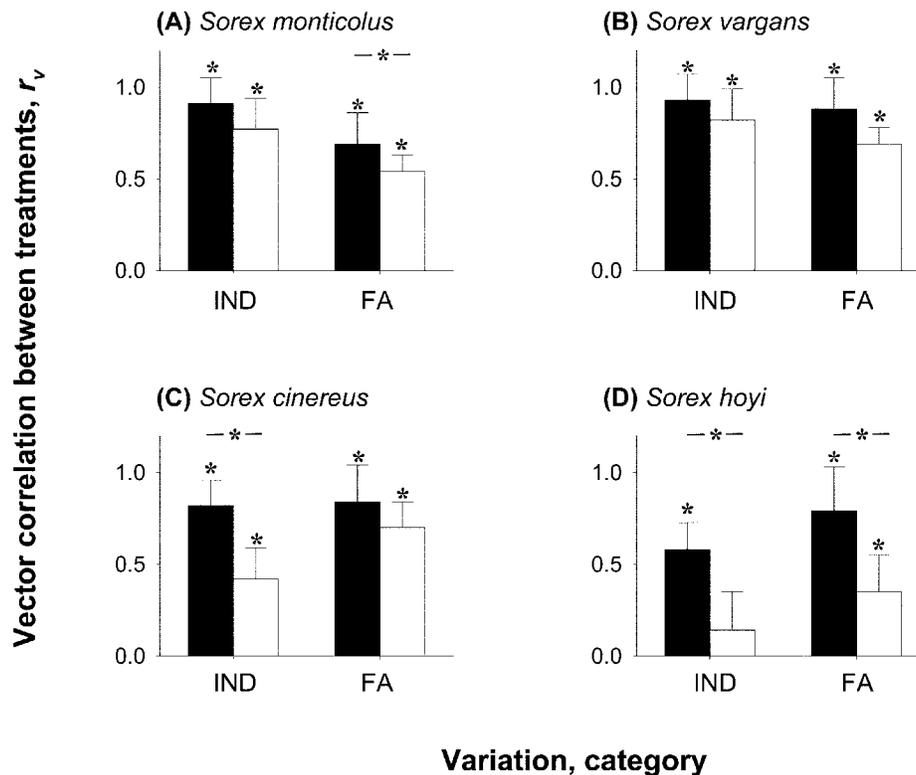
Figure 3: Vector correlations (means ± bootstrapped SD) between the first eigenvectors of landmark displacement due to among-individual variation (*IND*) under the control condition and among-individual variation under the vegetation removal treatment and between the first eigenvectors of landmark displacement due to fluctuating asymmetry (*FA*) under the control condition and fluctuating asymmetry under the vegetation removal treatment in (*A*) *Sorex monticolus*, (*B*) *Sorex vagrans*, (*C*) *Sorex cinereus*, and (*D*) *Sorex hoyi*. Black bars are "functionally integrated" landmarks; white bars are "nonintegrated" landmarks. Displacement of nonintegrated landmarks differs more between the treatments than displacement of functionally integrated landmarks. Asterisks over bars indicate that a correlation is significantly different from 0 ($t > 1.9$, $P = .05$); asterisks within a line show significant differences between treatments ($F > 2.38$, $P < .05$).

Stress-Induced Variation and Species Divergence

Displacement of landmarks that were parts of functional units was similar among species and among individuals within each species (r_v of among-species versus among-individual variation: integrated landmarks: 0.57 ± 0.19; nonintegrated landmarks: 0.12 ± 0.24; fig. 2; fig. 5A; figs. A1–A3). On the contrary, species differed the most in the displacement of the same (nonintegrated) landmarks that were most sensitive to the effects of treatment within each species (r_v of among species versus between treatments: integrated landmarks: 0.24 ± 0.18; nonintegrated landmarks: 0.62 ± 0.21; fig. 2; fig. 5B; figs. A1–A3).

Discussion

The modular structure of organisms enables adaptive modifications of some parts without interference with functionality of other parts (Simpson 1953; Raff 1996;

Wagner 2001). The patterns of morphological integration and modularity are produced by developmental interactions among traits and reflect most recurrent and strongest selection pressures (Berg 1960; Riska 1989; Gilbert et al. 1996; Kirschner and Gerhart 1998; Nijhout and Emlen 1998; von Dassow and Munro 1999). Thus, phenotypic variation available for external selection can be biased by selection for internal cohesiveness; developmental systems produce only a small subset of possible phenotypes (Alberch 1980; Chipman 2001; Arthur 2002). Correspondingly, existing patterns of integration often bias strongly the response of complex morphological structures to directional selection (e.g., Klingenberg and Leamy 2001). If accumulation of random developmental variation could facilitate adaptation of an organism to a novel environment as is sometimes suggested (Simons and Johnston 1997; Emlen et al. 2003; Hoffmann and Woods 2003), then such channeling of developmental errors by existing organismal structures might bias introduction of morpho-
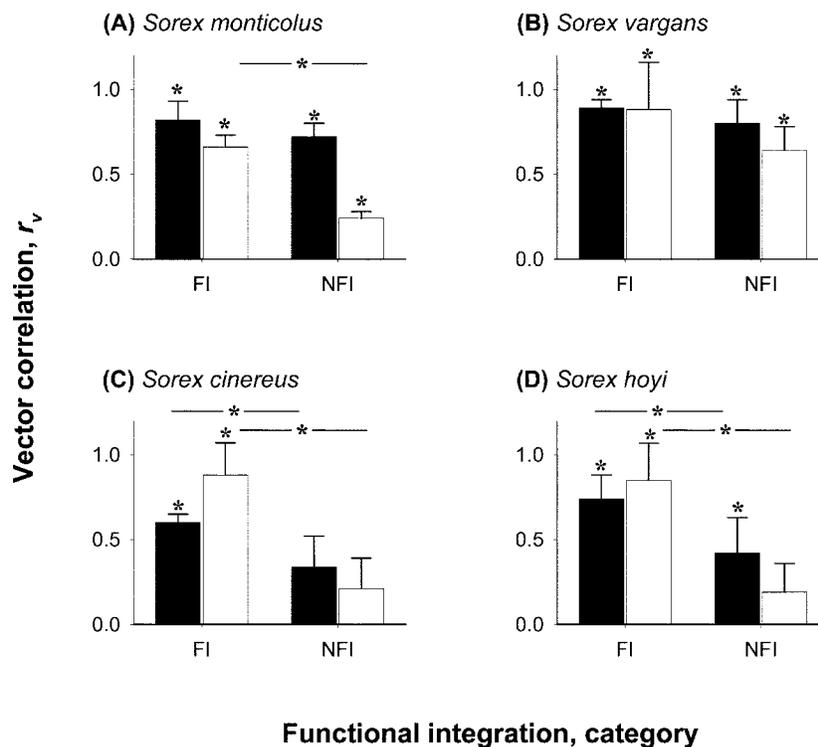
Figure 4: Vector correlations (means ± bootstrapped SD) between the first eigenvectors of landmark displacement due to among-individual variation and due to fluctuating asymmetry in (*A*) *Sorex monticolus*, (*B*) *Sorex vargans*, (*C*) *Sorex cinereus*, and (*D*) *Sorex hoyi*. Black bars are the control treatment; white bars are the vegetation removal treatment. *FI* designates functionally integrated landmarks; *NFI* designates nonintegrated landmarks. Similarity in among-individual variation and fluctuating asymmetry is generally higher for functionally integrated landmarks compared with nonintegrated landmarks. Asterisks over bars indicate that a correlation is significantly different from 0 ($t > 2.2$, $P < .05$); asterisks within a line show significant differences between displacements of integrated and nonintegrated landmarks under each treatment ($F > 2.36$, $P < .05$).

logical variation for external selection and thus influence evolutionary change.

Our study of morphological integration in among-individual variation and fluctuating asymmetries in shrews produced four principal results. First, all species had a strong integration of fluctuating asymmetries, especially in traits involved in the attachment of the same muscle. Second, among-individual variation was most similar with fluctuating asymmetry variation in functionally integrated traits compared with other traits. Third, despite a large (200%–300%) increase in the magnitude of fluctuating asymmetry in some traits under stress (Badyaev et al. 2000; Foresman and Badyaev 2003), the directionality and integration patterns of fluctuating asymmetries of these traits changed little, implying channeling effects of functional complexes on accumulation of developmental variation (see below). Finally, patterns of fluctuating asymmetry in the traits outside of functionally integrated units (non-integrated traits) were concordant with patterns of species difference in these traits, whereas functionally integrated traits were similar across species.

We found close integration of fluctuating asymmetries throughout the mandible; the integration was especially close in traits associated with the attachment of the same muscle (fig. 2; figs. A1–A3). Interestingly, in two species, integration of landmark displacement was higher under stressful conditions compared to control (figs. 2, 4), suggesting either strengthening of existing developmental links between the traits or the stress-induced coordination of development (see also Siegel and Doyle 1975; Graham et al. 2000; Emlen et al. 2003). Similarly, in bumblebees (*Bombus empatients*), fluctuating asymmetries of developmentally independent fore and hind wings became integrated only under stressful conditions but not under control conditions, apparently as a result of stress-induced resource exchange between the developmental modules (Klingenberg et al. 2001). More generally, congruence between the patterns of fluctuating asymmetry integration and a priori described functional units of a shrew mandible corroborated the results of other studies (Leamy 1993; Badyaev and Foresman 2000; Klingenberg and Zaklan 2000; Klingenberg 2003), showing that coordinated de-
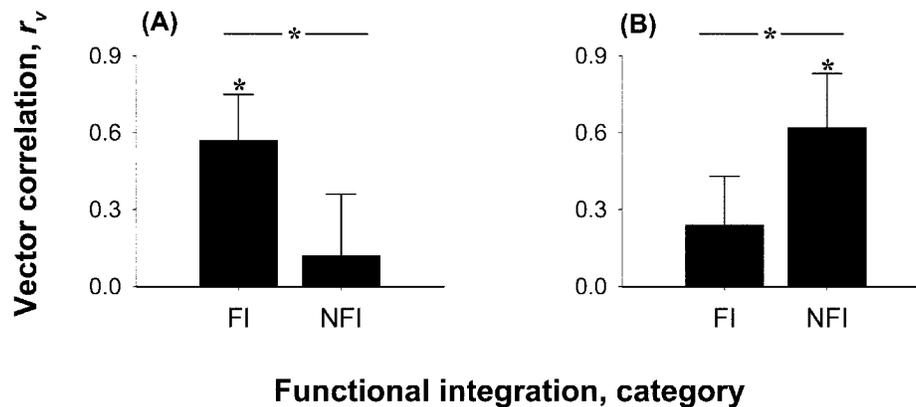
Figure 5: Vector correlations (means ± bootstrapped SD) between the first eigenvectors of landmark displacement due to (*A*) among-species variation and among-individual variation in functionally integrated (*FI*) and nonintegrated (*NFI*) landmarks (species are similar in patterns of functional integration) and (*B*) among-species variation and between treatments variation in FI and NFI landmarks (stress-induced variation in nonintegrated landmarks is similar to species divergence in these landmarks). Asterisks over bars indicate that correlations are significantly different from 0 (*A*: $t = 9.81$, $P < .01$; *B*: $t = 7.59$, $P < .01$); asterisks within a line show significant differences between displacements of integrated and nonintegrated landmarks (*A*: $F = 12.01$, $P < .01$; *B*: $F = 6.41$, $P < .05$).

velopment and function of morphological traits lead to their similarity in expression of developmental instabilities. Thus, in these species, the strength of integration among fluctuating asymmetries of morphological traits might be used to infer their integration during growth (Klingenberg 2003 [see also for notable exceptions]). It also follows that the magnitude of accumulation of developmental errors will be lower for integrated traits because of the compensatory and constraining interactions among developmentally linked components (Swaddle and Witter 1997; Hallgrimsson 1998; Aparicio and Bonal 2002; Badyaev 2003; Foresman and Badyaev 2003).

If developmental and functional interactions among morphological traits during growth result in coordinated expression of developmental errors by these traits, could the same developmental interactions account for among-individual trait variability? We found strong concordance of landmark displacement due to among-individual and fluctuating asymmetry (fig. 4). Similarly, patterns of fluctuating asymmetry were highly congruent with among-individual variation in several studies of insect and bat wings and mammalian lower jaws (Leamy 1993; Klingenberg and McIntyre 1998; Klingenberg and Zaklan 2000; Juste et al. 2001*a*, 2001*b*; Klingenberg et al. 2001). Congruence in the among-individual and within-individual (fluctuating asymmetry) patterns of variation suggest that these distinct sources of variation are expressed by the same developmental pathways (Cheverud 1982; Debat and David 2001), a pattern identified by some recent theoretical studies (Wagner et al. 1997; Meiklejohn and Hartl 2002; Siegal and Bergman 2002; Badyaev 2004; see also

Ancel and Fontana 2000). On the contrary, Debat et al. (2000) found dissimilar patterns of morphological variation between individual variation and fluctuating asymmetry and suggested that the concordance documented in other studies is a result of functional integration in structures for which bilateral symmetry itself is of crucial functional importance (see also Breuker and Brakefield 2003).

Some aspects of the biology of shrew species in this study make it probable that similar developmental processes might produce concordance between individual variation and fluctuating asymmetry. First, in these species, ossification of the skull and lower jaw and corresponding formation of functional muscle complexes coincide with the onset of independent foraging (Foresman 1994). Thus, characteristics of local prey can determine the necessary bite force and corresponding patterns of integration in the still cartilaginous lower jaw. Second, shrews are unusual among mammals in having an unfused mandibular symphysis that allows independent movement of the left and right jaw and enables coordination between each jaw during prey handling (Dötsch 1982); that is, the lower left and right incisors can be used as forceps to hold prey. Thus, close functional coordination between the left and right jaw during growth can provide an additional source of integration of fluctuating asymmetries between the sides and of concordance between among-individual variation and fluctuating asymmetry. The role of the mandibular symphysis in enabling developmental and functional integration between the sides of the jaw and corresponding integration of developmental errors was also documented in other mammals (Tuominen et al. 1993; Legrell and

Isberg 1999). Finally, the short generation time of shrews (life span 12–18 mo) in relation to the environmental seasonality of our study sites and their limited natal dispersal might favor maintenance of sensitivity to local environmental variation during growth. Controlled experiments are needed to partition the effects of prey characteristics, within-jaw functional integration, and developmental stress documented in this study.

The species in this study were most similar in functionally integrated traits (that showed less sensitivity to environmental stress) and most different in nonintegrated traits (that were the most responsive to stress within species; fig. 5). These observations raise two questions. First, how do we reconcile apparently strong environmental determination of functional complexes during ontogeny with their long historical persistence? Second, how can the traits that are most sensitive to environmental stress during ontogeny nevertheless contribute to, and apparently bias, species divergence? (fig. 5).

Increased morphological variation in nonintegrated tissues under stressful conditions can create an opportunity for adaptive changes in bite force that are favored in novel environments. Modification of bite force favored under altered environmental conditions is often enabled by changes in tissues located between muscle attachment points (and thus determining the force exerted by a muscle; Dötsch 1982, 1986; Carraway and Verts 1994) without the changes in the muscle attachments themselves. We found that stress-induced variation was mostly confined to nonintegrated tissues located between muscle attachment complexes (see also Foresman and Badyaev 2003). Similarly, Mabee et al. (2000) found that the effects of environmental variation were mostly confined to nonintegrated cartilaginous traits and that the effects of stress on morphological integration depended on its timing in relation to ossification (see also Zelditch and Carmichael 1989; Vasil'eva 1997).

In this study, shrews that were born under stressful treatment experienced two kinds of stresses—early nonspecific and late-specific stresses in relation to the timing and development of functional integration in the lower jaw. First, mothers were in lower condition during gestation and lactation, which probably had a negative impact on early development of offspring; offspring born under stressful treatments had more developmental abnormalities and weighed less (Badyaev et al. 2000). Second, overstory removal altered the composition of local prey and increased food competition compared with the previous generation. This effect became important once juveniles started foraging on their own. Muscle attachments themselves form before weaning, and therefore environmental effects associated with independent foraging might be confined to the later ossified and nonintegrated tissues of the jaw (see

also Zelditch et al. 1992, 1993). Thus, historical persistence of functional complexes might be enabled by a mosaic pattern of development in the lower jaw where local adjustments of bite force are accommodated by environmentally sensitive tissues located between muscle attachment complexes.

In shrews, periods of environmental stress caused by habitat alteration are often accompanied by increased food competition, extensive mortality, and changes in population distribution (Zakharov et al. 1991; Pankakoski et al. 1992). An increase in morphological variation that accompanies environmental stress can be beneficial if novel modification of the foraging apparatus facilitates exploitation of previously unavailable prey. Such stress-induced beneficial changes (here confined to nonintegrated and later ossified tissues) might persist in small and isolated populations, leading to rapid divergence of populations in ecologically important traits (Emlen et al. 2003). Thus, similarity of species in nonintegrated traits that are most sensitive to stress within each species can be explained in two ways. First, large stress-induced changes in nonintegrated tissues might enable rapid divergence without changes in functionally integrated components of foraging apparatus. Second, both environmental and genetic variation can be expressed by the same developmental mechanisms, making nonintegrated traits in the mandible more sensitive not just to environmental variation but also to recurrent and fluctuating directional selection as well as to genetic drift (see also Ancel and Fontana 2000; Badyaev 2004). Overall, the strong effect of functional complexes on directing and incorporating stress-induced variation during growth can contribute to the historical persistence of functionally integrated sets of traits in shrew foraging apparatus despite their high sensitivity to environmental variation.

Literature Cited

Alberch, P. 1980. Ontogenesis and morphological diversification. American Zoologist 20:653–667.

Ancel, L. W., and W. Fontana. 2000. Plasticity, evolvability,

and modularity in RNA. Journal of Experimental Zoology 288:242–283.

Aparicio, J. M., and R. Bonal. 2002. Why do some traits show higher fluctuating asymmetry than others? a test of hypotheses with tail feathers of birds. Heredity 89: 139–144.

Ärnbäck-Christie-Linde, A. 1907. Der Bau der Sociciden und ihre Beziehungen zu andern Säugetieren. Gegenbaurs Morphologisches Jahrbuch 36:463–514.

Arthur, W. 2001. Developmental drive: an important determinant of the direction of phenotypic evolution. Evolution and Development 3:271–278.

———. 2002. The emerging conceptual framework of evolutionary developmental biology. Nature 415:757–764.

Arthur, W., and M. Farrow. 1999. The pattern of variation in centipede segment number as an example of developmental constraint in evolution. Journal of Theoretical Biology 200:183–193.

Baatz, M., and G. P. Wagner. 1997. Adaptive inertia caused by hidden pleiotropic effects. Theoretical Population Biology 51:49–66.

Badyaev, A. V. 2002. Growing apart: an ontogenetic perspective on the evolution of sexual size dimorphism. Trends in Ecology & Evolution 17:369–378.

———. 2003. Integration and modularity in the evolution of sexual ornaments: an overlooked perspective. Pages 50–79 *in* M. Pigliucci and K. Preston, eds. The evolutionary biology of complex phenotypes. Oxford University Press, Oxford.

———. 2004. Role of stress in evolution: from individual adaptability to evolutionary adaptation. *In* B. Hallgrimsson and B. K. Hall, eds. Variation: a hierarchical examination of a central concept in biology. Academic Press, San Diego.

Badyaev, A. V., and K. R. Foresman. 2000. Extreme environmental change and evolution: stress-induced morphological variation is strongly concordant with patterns of evolutionary divergence in shrew mandibles. Proceedings of the Royal Society of London B 267:371–377.

Badyaev, A. V., and R. L. Young. 2004. Complexity and integration in sexual ornamentation: an example with carotenoid and melanin plumage pigmentation. Journal of Evolutionary Biology 17 (in press).

Badyaev, A. V., K. R. Foresman, and M. V. Fernandes. 2000. Stress and developmental stability: vegetation removal causes increased fluctuating asymmetry in shrews. Ecology 81:336–345.

Beldade, P., K. Koops, and P. M. Brakefield. 2002. Developmental constraints versus flexibility in morphological evolution. Nature 416:844–846.

Berg, R. L. 1960. The ecological significance of correlation pleiades. Evolution 14:171–180.

Brakefield, P. M. 2003. Artificial selection and the development of ecologically relevant phenotypes. Ecology 84: 1661–1671.

Breuker, C. J., and P. M. Brakefield. 2003. Lack of response to selection for lower fluctuating asymmetry of mutant eyespots in the butterfly *Bicyclus anynana.* Heredity 91: 17–27.

Carraway, L. N., and B. J. Verts. 1994. Relationship of mandibular morphology to relative bite force in some *Sorex* from western North America. Pages 201–210 *in* J. F. Merritt, G. L. J. Kirkland, and R. K. Rose, eds. Advances in the biology of shrews. Carnegie Museum of Natural History, Pittsburgh.

Cheverud, J. M. 1982. Phenotypic, genetic, and environmental morphological integration in the cranium. Evolution 36:499–516.

Chipman, A. 2001. Developmental exaptation and evolutionary change. Evolution and Development 3:299–301.

Dannelid, E. 1994. Comparison of pigment and other dental characters of eastern Palearctic *Sorex* (Mammalia: Soricidae). Pages 217–231 *in* J. F. Merritt, G. L. J. Kirkland, and R. K. Rose, eds. Advances in the biology of shrews. Carnegie Museum of Natural History, Pittsburgh.

Debat, V., and P. David. 2001. Mapping phenotypes: canalization, plasticity and developmental stability. Trends in Ecology & Evolution 16:555–561.

Debat, V., P. Alibert, P. David, E. Paradis, and J.-C. Auffray. 2000. Independence between developmental stability and canalization in the skull of the house mouse. Proceedings of the Royal Society of London B 267:423–430.

Dötsch, C. 1982. Der Kauapparat der Sociidae (Mammalia Insectivora). Funktionsmorphologische Untersuchungen zur Kaufunktion bei Spitzmäusen der Gattungen *Sorex* L., *Neomys* K., and *Crocidura*. Zoologischen Jahrbuchern (Anatomie) 108:421–484.

———. 1986. Mastication in the musk shrew, *Suncus murinus* (Mammalia, Soricidae). Journal of Morphology 189:25–43.

Dworkin, I. M., S. Tanda, and E. Larsen. 2001. Are entrenched characters developmentally constrained? creating biramous limbs in an insect. Evolution and Development 3:424–431.

Emlen, D. J., and H. F. Nijhout. 2000. The development and evolution of exaggerated morphologies in insects. Annual Reviews in Entomology 45:661–708.

Emlen, J. M., D. C. Freeman, and J. H. Graham. 2003. The adaptive basis of developmental instability: a hypothesis and its implication. Pages 51–61 *in* M. Polak, ed. Developmental instability: causes and consequences. Oxford University Press, Oxford.

Foresman, K. R. 1994. Comparative embryonic develop-

ment of the Soricidae. Pages 241–258 *in* J. F. Merritt, G. L. J. Kirkland, and R. K. Rose, eds. Advances in the biology of shrews. Carnegie Museum of National History, Pittsburgh.

Foresman, K. R., and A. V. Badyaev. 2003. Developmental instability and the environment: why are some species better indicators of stress than others? *In* J. F. Meritt, G. L. J. Kirkland, and R. K. Rose, eds. Advances in the biology of shrews. Carnegie Museum of Natural History, Pittsburgh.

Fusco, G. 2001. How many processes are responsible for phenotypic evolution? Evolution and Development 3: 279–286.

Gilbert, S. F., J. M. Opitz, and R. A. Raff. 1996. Resynthesizing evolutionary and developmental biology. Developmental Biology 173:357–372.

Goodall, C. 1991. Procrustes methods in the statistical analysis of shape. Journal of Royal Statistics Society B 53:285–339.

Graham, J. H., D. Fletcher, J. Tigue, and M. McDonald. 2000. Growth and developmental stability of *Drosophila melanogaster* in low frequency magnetic fields. Bioelectromagnetics 21:465–472.

Hallgrimsson, B. 1998. Fluctuating asymmetry in the mammalian skeleton: evolutionary and developmental implications. Evolutionary Biology 30:187–251.

———. 1999. Ontogenetic patterning of skeletal fluctuating asymmetry of rhesus macaques and human: evolutionary and developmental implications. International Journal of Primatology 20:121–151.

Helm, J. W., and R. Z. German. 1996. The epigenetic impact of weaning on craniofacial morphology during growth. Journal of Experimental Zoology 276:243–253.

Hoffmann, A. A., and R. E. Woods. 2003. Associating environmental stress with developmental stability: problems and patterns. Pages 387–401 *in* M. Polak, ed. Developmental instability: causes and consequences. Oxford University Press, Oxford.

Jablonka, E., and M. J. Lamb. 1995. Epigenetic inheritance and evolution: the Lamarkian perspective. Oxford University Press, Oxford.

Juste, J., C. Lopez-Gonzalez, and R. E. Strauss. 2001*a*. Analysis of asymmetries in the African fruit bats *Eidolon helvum* and *Rousettus egyptiacus* (Mammalis: Magachiroptera) from the islands of the Gulf of Guinea. I. Variance and size components of bilateral variation. Journal of Evolutionary Biology 14:663–671.

———. 2001*b*. Analysis of asymmetries in the African fruit bats *Eidolon helvum* and *Rousettus egyptiacus* (Mammalis: Magachiroptera) from the islands of the Gulf of Guinea. II. Integration and levels of multivariate fluctuating asymmetry across a geographical range. Journal of Evolutionary Biology 14:672–680.

Kindahl, M. 1959. Some aspects of the tooth development in Soricidae. Acta Odontologica Scandinavica 17:203–237.

Kirschner, M., and J. Gerhart. 1998. Evolvability. Proceedings of the National Academy of Sciences of the USA 95:8420–8427.

Klingenberg, C. P. 2003. Developmental instability as a research tool: using patterns of fluctuating asymmetry to infer the developmental origins of morphological integration. Pages 427–442 *in* M. Polak, ed. Developmental instability: causes and consequences. Oxford University Press, Oxford.

Klingenberg, C. P., and L. Leamy. 2001. Quantitative genetics of geometric shape in the mouse mandible. Evolution 55:2342–2352.

Klingenberg, C. P., and G. S. McIntyre. 1998. Geometric morphometrics of developmental instability: analyzing patterns of fluctuating asymmetry with Procrustes methods. Evolution 53:1363–1375.

Klingenberg, C. P., and H. F. Nijhout. 1998. Competition among growing organs and developmental control of morphological asymmetry. Proceedings of the Royal Society of London B 265:1135–1139.

Klingenberg, C. P., and S. D. Zaklan. 2000. Morphological integration among developmental compartments in the *Drosophila* wing. Evolution 54:1273–1285.

Klingenberg, C. P., A. V. Badyaev, S. M. Sowry, and N. J. Beckwith. 2001. Inferring developmental modularity from morphological integration: analysis of individual variation and asymmetry in bumblebee wings. American Naturalist 157:11–23.

Leamy, L. J. 1993. Morphological integration of fluctuating asymmetry in the mouse mandible. Genetica 89:139–153.

Legrell, P. E., and A. Isberg. 1999. Mandibular length and midline asymmetry after experimentally induced temporomandibular joint disk displacement in rabbits. American Journal of Orthodontics and Dentofacial Orthopedics 115:247–253.

Lively, C. M. 1986. Canalization versus developmental conversion in a spatially variable environment. American Naturalist 128:561–572.

Mabee, P. M., K. L. Olmstead, and C. C. Cubbage. 2000. An experimental study of intraspecific variation, developmental timing, and heterochrony in fishes. Evolution 54:2091–2106.

Meiklejohn, C. D., and D. L. Hartl. 2002. A single mode of canalization. Trends in Ecology & Evolution 17:468–473.

Muller, G. B. 2003. Embryonic motility: environmental influences and evolutionary innovation. Evolution and Development 5:56–60.

Nijhout, H. F., and D. J. Emlen. 1998. Competition among

body parts in the development and evolution of insect morphology. Proceedings of the National Academy of Sciences of the USA 95:3685–3689.

Olson, E. C., and R. L. Miller. 1958. Morphological integration. University of Chicago Press, Chicago.

Pankakoski, E., I. Koivisto, and H. Hyvärinen. 1992. Reduced developmental stability as an indicator of heavy metal pollution in the common shrew *Sorex araneus*. Acta Zoologica Fennica 191:137–144.

Raff, R. A. 1996. The shape of life: genes, development, and the evolution of animal form. University of Chicago Press, Chicago.

Riska, B. 1986. Some models for development, growth, and morphometric correlation. Evolution 40:1303–1311.

———. 1989. Composite traits, selection response, and evolution. Evolution 43:1172–1191.

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

Salazar-Ciudad, I., and J. Jernvall. 2002. A gene network model accounting for development and evolution of mammalian teeth. Proceedings of the National Academy of Sciences of the USA 99:8116–8120.

Schlichting, C. D., and M. Pigliucci. 1998. Phenotypic evolution: a reaction norm perspective. Sinauer, Sunderland, Mass.

Schmalhausen, I. I. 1949. Factors of evolution. Blakiston, Philadelphia.

Siegal, M. L., and A. Bergman. 2002. Wadington's canalization revisited: developmental stability and evolution. Proceedings of the National Academy of Sciences of the USA 99:10528–10532.

Siegel, M. I., and W. J. Doyle. 1975. The differential effects of prenatal and postnatal audiogenic stress on fluctuating dental asymmetry. Journal of Experimental Zoology 191:211–214.

Simons, A. M. 2002. The continuity of microevolution and macroevolution. Journal of Evolutionary Biology 15:688–701.

Simons, A. M., and M. O. Johnston. 1997. Developmental instability as a bet-hedging strategy. Oikos 80:401–405.

Simpson, G. G. 1953. The major features of evolution. Simon & Schuster, New York.

Stern, D. L., and D. J. Emlen. 1999. The developmental basis for allometry in insects. Development 126:1091–1101.

Swaddle, J. P., and M. S. Witter. 1997. On the ontogeny

of developmental stability in a stabilized trait. Proceedings of the Royal Society of London B 264:329–334.

Tuominen, M., T. Kantomaa, and P. Pirttiniemi. 1993. Effect of food consistency on the shape of the articular eminence and the mandible: an experimental study on the rabbit. Acta Odontologica Scandinavica 51:65–72.

Vasil'eva, E. D. 1997. Size-dependent variability and sexual dimorphism of skull in teleostean fishes: determining factors and main trends. Voprosy Ikhtiologii 37:293–306.

von Dassow, G., and E. Munro. 1999. Modularity in animal development and evolution: elements of a conceptual framework for EvoDevo. Journal of Experimental Zoology 285:307–325.

Wagner, G. P. 2001. The character concept in evolutionary biology. Academic Press, New York.

Wagner, G. P., and L. Altenberg. 1996. Complex adaptation and the evolution of evolvability. Evolution 50:967–976.

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

Waxman, D., and J. R. Peck. 1998. Pleiotropy and the preservation of perfection. Science 279:1210–1213.

West-Eberhard, M. J. 1989. Phenotypic plasticity and the origins of diversity. Annual Review of Ecology and Systematics 20:249–278.

———. 2003. Developmental plasticity and evolution. Oxford University Press, Oxford.

Whyte, L. L. 1965. Internal factors in evolution. Braziller, New York.

Zakharov, V. M., E. Pankakoski, B. I. Sheftel, A. Prltonen, and I. Hanski. 1991. Developmental stability and population dynamics in the common shrew, *Sorex araneus*. American Naturalist 138:797–810.

Zarkower, D. 2001. Establishing sexual dimorphism: conservation amidst diversity? Nature Reviews Genetics 2:175–185.

Zelditch, M. L., and A. C. Carmichael. 1989. Ontogenetic variation in patterns of developmental and functional integration in skulls of *Sigmodon fulviventer*. Evolution 43:814–824.

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

———. 1993. The ontogenetic complexity of developmental constraints. Journal of Evolutionary Biology 6:621–641.