

EVOLUTIONARY PERSISTENCE OF PHENOTYPIC INTEGRATION: INFLUENCE OF DEVELOPMENTAL AND FUNCTIONAL RELATIONSHIPS ON COMPLEX TRAIT EVOLUTION

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Abstract.—Examination of historical persistence of integration patterns provides an important insight into understanding the origin and evolution of complex traits. Specifically, the distinct effects of developmental and functional integration on the evolution of complex traits are often overlooked. Because patterns of functional integration are commonly shaped by selection exerted by the external environment, whereas patterns of developmental integration can be determined by relatively environment-independent selection for developmental homeostasis, examination of historical persistence of morphological integration patterns among species should reveal the relative importance of current selection in the evolution of complex traits. We compared historical persistence of integration patterns produced by current developmental versus ecological requirements by examining the evolution of complex mandibular structures in nine species of soricid shrews. We found that, irrespective of phylogenetic relatedness of species, patterns of developmental and functional integration were highly concordant, suggesting that strong selection for developmental homeostasis favors concordant channeling of both internal and external variation. Overall, our results suggest that divergence in mandible shape among species closely follows variation in functional demands and ecological requirements regardless of phylogenetic relatedness among species.

Key words.—Fluctuating asymmetry, geometric morphometrics, mandible, morphological integration, *Sorex*.

Received September 13, 2005. Accepted April 12, 2006.

A fundamental question in evolutionary biology is how complex structures evolve (Riska 1989; Raff 1996). Both developmental and functional requirements produce correlations among traits, and these correlations can strongly affect the evolutionary trajectory of complex structures (Wake et al. 1983; Arthur 2001). Yet, it is unclear whether integrated structures that are the units of development and units of selection are also the units of evolution.

Historical persistence of integration patterns should result from historical constancy of selection operating during trait development or function (Lande 1980; Cheverud 1982, 1984, 1995, 1996). However, the empirical evidence for historical persistence of integration patterns is inconsistent. In some systems integration patterns are remarkably constant across species (e.g., Huber and Stuefer 1997; Preston and Ackerly 2004), whereas recent experimental studies have shown that strong selection for a novel phenotype and novel integration patterns, rapidly override pre-existing developmental relationships among traits (Beldade et al. 2002; Brakefield 2003). This indicates that patterns of integration may vary across environments (Preston and Ackerly 2004) and among closely related species (Schwenk and Wagner 2001, 2004).

Both developmental and functional relationships contribute to overall patterns of morphological integration (Olson and Miller 1958; Cheverud 1996). Yet, it is rarely examined whether the source of trait correlations (i.e., development or function) affects the historical persistence of an integration pattern. Developmental integration is produced when components of a complex structure share common developmental precursors, pathways, or resources and it can be environment independent (Riska 1986; Atchley and Hall 1991; Cheverud 1995; Raff 1996; Klingenberg and Nijhout 1998; Klingenberg et al. 2001; Schwenk 2001; Hall 2003; Hallgrímsson et al. 2003; Badyaev 2004; Badyaev and Young 2004). However,

this conventional definition of developmental integration also includes correlations among traits that result from a similar response by individuals or species to comparable selection pressures and, thus, may reflect common evolutionary history, promoting the view that developmental constraints are equivalent to phylogenetic constraints (Lauder 1981; “local constraints” sensu Smith et al. 1985; Gould 1989; McKittrick 1993; Watson et al. 1995; West-Eberhard 2003). Functional integration, on the other hand, occurs when multiple traits must interact to perform an organismal function favoring particular functional associations among traits (Cheverud 1996; Badyaev and Foresman 2000; Schwenk and Wagner 2001; Monteiro et al. 2005). These functional associations are defined by current natural selection, and thus vary across environments (Schwenk 2001). However, because persistent selection for functional relationships among traits favors the evolution of developmental integration (Olson and Miller 1958; Cheverud 1982, 1995), descriptions of morphological integration patterns may include both developmental and functional relationships. Thus, because patterns of phylogenetic constraints, developmental integration, and functional integration may overlap, examination of evolutionary persistence of developmental and functional integration requires isolation of these three factors.

To distinguish current developmental interrelationships among traits from phylogenetic constraints, developmental integration can be measured as covariation in fluctuating asymmetries (FA) of components of a complex structure. Trait variation due to FA results from random perturbations during development and is expected to be randomly distributed unless traits share direct developmental relationships (Riska 1986; Klingenberg 2003; Klingenberg et al. 2003; Badyaev and Foresman 2004; Badyaev et al. 2005). Thus, FA covariation should reflect patterns of developmental in-

tegration, and persistence of developmental integration of FA across species is expected only when selection for developmental homeostasis is similar among species and environments. Isolating developmental and functional relationships requires knowledge of how the local environment influences trait development and function separately. In *Sorex* shrews the ossification of the foraging apparatus is delayed and coincides with initiation of independent foraging (Foresman 1994). Thus, in these species, functional integration of the muscle attachment areas reveals functional requirements of foraging in a particular environment. In this case, persistence of functional integration across species is expected when species share similar ecological requirements (e.g., diet).

Here, we examine the historical persistence of developmental and functional integration in complex morphological structures among nine species of soricid shrews (Fig. 1). We predict that if internal selection for developmental homeostasis has a stronger effect than selection for function on patterns of morphological integration, then between-species congruence of integration will be consistent with phylogeny, highlighting the importance of common evolutionary history for morphological divergence in shrews. Alternatively, if selection for function in current environment had stronger effect than internal selection on historical morphological divergence, then between-species congruence of integration will be inconsistent with the patterns expected from phylogenetic relationships among species, such that species experiencing similar functional demands will have similar morphologies regardless of phylogenetic relatedness.

MATERIALS AND METHODS

Data Collection

In nine species of *Sorex* shrews (Fig. 1), we measured mandibles of fully grown individuals: *S. cinereus* ($n = 18$), *S. fumeus* ($n = 19$), *S. haydeni* ($n = 18$), *S. hoyi* ($n = 18$), *S. monticolus* ($n = 18$), *S. pacificus* ($n = 18$), *S. palustris* ($n = 20$), *S. trowbridgii* ($n = 19$), and *S. vagrans* ($n = 18$). Left and right mandibles were separated and placed on a slide and photographed at high resolution using a five-megapixel digital camera (Camedia E-20 Olympus, Tokyo) mounted in a standard position, photographed under $10\times$ magnification using a Leica (Bannockburn, IL) DC 300, or photographed under $7.5\times$ magnification using an Olympus SZH stereo photomicroscope and video-capture board (for more detailed protocol, see Badyaev and Foresman 2000, 2004). Analyses of all images were conducted using SigmaScan 5.0 Pro software (SPSS Inc., Chicago, IL).

We obtained x - and y -coordinates from 15 homologous morphological landmarks, commonly used in studies of shrew mandibles (Kindahl 1959; Dannelid 1998; Badyaev and Foresman 2000, 2004; Badyaev et al. 2000; Fig. 2A). We scaled images to standard size using rulers photographed with mandibles and examined repeatability of all coordinates. Only landmarks with greater than 97.5% repeatability were included in the study. Repeatability was calculated from the intraclass correlation coefficient (Lessels and Boag 1987) of ANOVA from a subset of 23 individuals (at least two from each species) measured multiply.

To describe developmental integration, we examined cor-

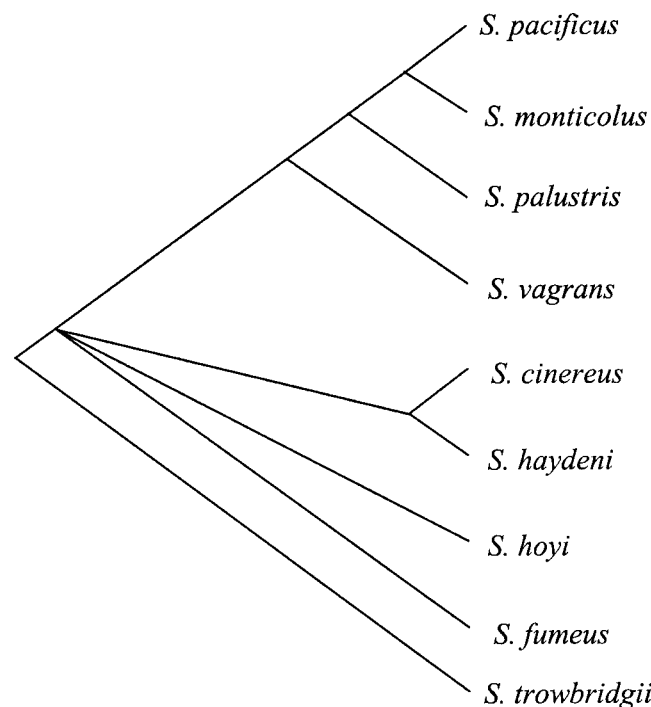


FIG. 1. Phylogeny of nine species of *Sorex* shrews used in this study (based on Fumagalli et al. 1999).

relations of FA in the mandible. FA was calculated for all x - and y -coordinates as the left minus the right coordinate. ANOVA results of left minus right values revealed that asymmetries of landmarks 8 and 14 (Fig. 2) differed significantly from zero, indicating directional asymmetry rather than FA; thus, these landmarks were not included in the analyses. To describe functional integration, we analyzed landmarks associated with muscle and connective tissue attachment in the shrew mandible, any landmark within these regions was considered functionally integrated (ellipsoids in Fig. 2A). Selection of functionally integrated landmarks was based on dissections (Badyaev et al. 2005), anatomical records (Gaughran 1954), and previous literature on shrew mandible morphology (Dannelid 1998; Reumer 1998; Badyaev and Foresman 2000, 2004).

Data Analysis

Mandible shape variation

To remove variation due to mandible size, we first reflected left mandibles to their mirror image by assigning a negative to the x -coordinate of each landmark. All specimens were subsequently scaled to unit centroid size and landmark configurations were aligned from all landmarks, species, individuals, and body sides (after Klingenberg and McIntyre 1998; Badyaev and Foresman 2000, 2004) using a single Procrustes superimposition (generalized orthogonal least-squares fit; Rohlf and Slice 1990).

Variation of landmark configurations (Procrustes coordinates) was partitioned with ANOVA (Goodall 1991). Individual identity was nested within species and entered as a random effect, while mandible side was entered as a fixed

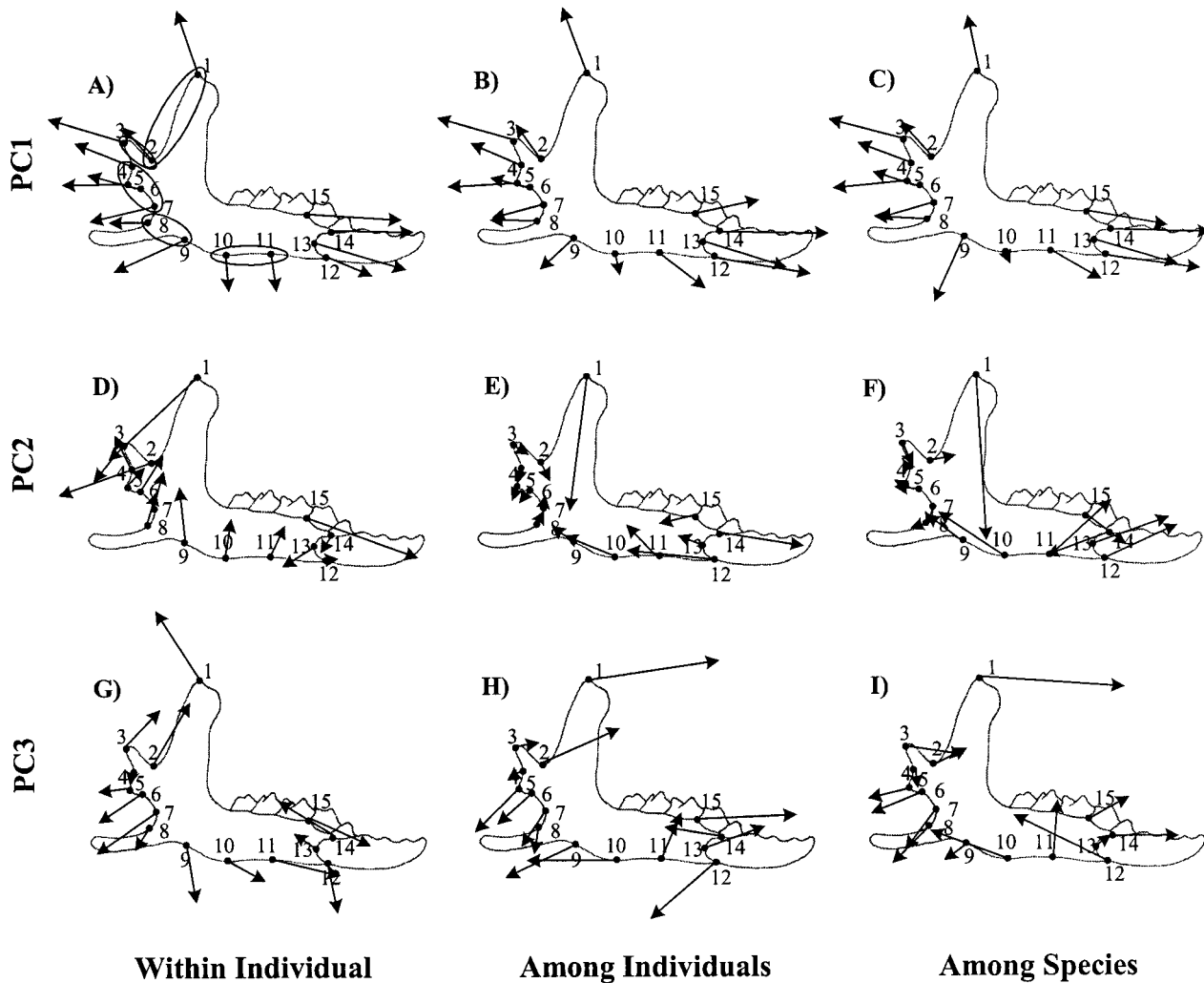


FIG. 2. Fifteen homologous landmarks describing mandible shape. (A–C) First principal components (PC1) of the covariance matrix of landmark displacement due to variation within individual (i.e., fluctuating asymmetry), among-individuals, and among-species respectively. (D–F) Second principal components (PC2) of the covariance matrix of landmark displacement due to variation within individual, among individuals, and among-species. (G–I) Third principal components (PC3) of the covariance matrix of landmark displacement due to variation within individual, among-individuals, and among species. Vector length is multiplied by 10 for better visibility. Ellipsoids indicate functionally integrated landmarks (i.e., muscle or connective tissue attachment sites).

effect. To assess contribution of each landmark to overall variation in mandible shape, x and y mean squares (MS) of each landmark were summed and variance components of MS for each effect were computed (Klingenberg and McIntyre 1998). To visualize the displacement of each landmark due to each effect in the model, we analyzed the covariance matrices calculated based on the expected MS matrices of sums of squares and cross products for species, individual, and side (Klingenberg and McIntyre 1998; Badyaev and Foresman 2000; Debat et al. 2000; Klingenberg and Zaklan 2000). We calculated the first three principal components (PC1–3) of each effect and plotted the loading for each PC and landmark as the displacement of each effect from the consensus position.

To test for patterns of landmark displacements across effects (within-individual, among-individuals, and among-species variation), we evaluated consistency of landmark displacements within and outside of functional units by calcu-

lating vector angles of within-individual and among-individual variation as well as variation among individuals and among species. A vector angle is the angle between two PC and was calculated as:

$$\Theta = \arccos [\mathbf{A} \cdot \mathbf{B} / (|\mathbf{A}| \cdot |\mathbf{B}|)], \quad (1)$$

where \mathbf{A} and \mathbf{B} are vectors containing the PC eigenvectors of each shape coordinate, and $|\mathbf{A}|$ and $|\mathbf{B}|$ are the length of \mathbf{A} and \mathbf{B} ; a more acute angle indicates greater similarity. We calculated vector angles for each pair of the first three PCs individually and plotted arcsine-transformed vector angles and bootstrapped standard deviations (Fig. 3; Sokal and Rohlf 1995). We determined differences in the consistency of variation within and outside of functional units using a t -test.

To describe developmental and functional integration, we conducted PC analysis for each species on FA values of all Procrustes coordinates and Procrustes coordinates of all functional landmarks, respectively. To assess reliability of the

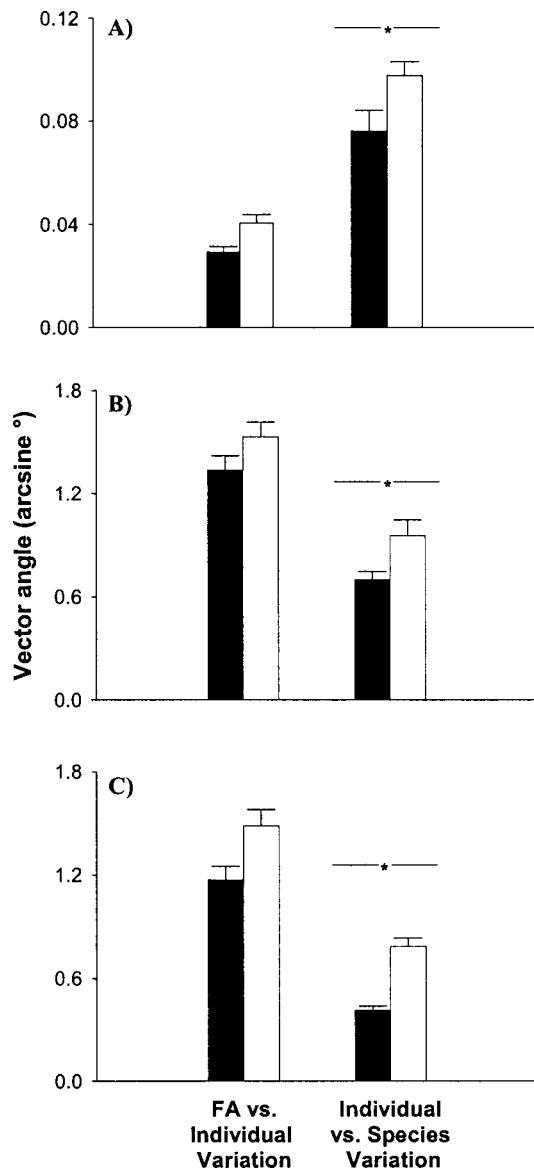


FIG. 3. Arcsine-transformed vector angles (mean \pm bootstrapped SD) of the landmark displacements for within-individual (fluctuating asymmetry) variation and among-individual variation in functionally integrated landmarks versus nonfunctionally integrated landmarks and among-individual and among-species variation in functionally integrated landmarks versus nonfunctionally integrated landmarks for (A) PC1, (B) PC2, and (C) PC3. Solid bars indicate comparisons of landmark displacements within functional units, open bars indicated comparison of landmark displacements outside of functional units. Smaller vector angles indicate greater congruence in direction and magnitude of landmark displacement. Landmarks displacements were more similar within than outside of functional units in comparisons among individuals and species and tended to be more similar in comparisons within and among individuals. Asterisk indicates statistical significance ($P < 0.05$).

PCs for each species we sampled with replacement ($n = 1000$ replicates) from x and y Procrustes coordinates, recalculated PCs, and compared observed eigenvalues of the first three PCs to the bootstrapped distribution of eigenvalues. All observed PCs were distinct from the bootstrapped distribution.

Persistence of developmental and functional integration

We quantified interspecific similarity of developmental integration as the vector angle between PC1 of each species pair, PC2 of each species pair, and PC3 of each species pair. Interspecific concordance of functionally integrated landmarks (ellipsoids in Fig. 2A) was calculated as vector angles of PC1 of functional landmarks for each species pair; the same comparisons were done for PC2 and PC3. Confidence intervals and significance of vector angles across species pairs were obtained by sampling with replacement from x - and y -coordinates ($n = 1000$ replicates) and recalculating vector angles.

To test for congruence of phylogenetic relatedness and patterns of developmental and functional integration, each species pair was given a rank relatedness determined by the number of internal nodes between the species (based on phylogeny of Fumagalli et al. 1999). Between-species arcsine-transformed vector angles (Sokal and Rohlf 1995) for the first three PCs were then plotted in relation to this rank (Fig. 4). This method assumes independence of function and speciation, such that more closely related species are assumed to be no more similar in function than distantly related species. When this assumption is not met, this method may overestimate consistency of between species vector angles for functional integration and phylogenetic relatedness. In *Sorex*, variation in foraging strategies and habitat preferences vary among species independently of phylogenetic relatedness (R. L. Young and A. V. Badyaev, unpubl. ms.). To test for a relationship between patterns of integration and phylogenetic relatedness among species, individual's species affiliation was shuffled ($n = 1000$ replicates) and vector angles were recalculated; significance was determined by testing for homogeneity of slopes between the observed relationship of patterns of developmental and functional integration and phylogenetic relatedness to the simulated relationship (Sokal and Rohlf 1995).

RESULTS

Mandible Shape Variation

Extent and patterns of mandible shape variation differed across the first three PCs of the covariance matrix of landmark displacements due to within-individual, among-individual, and among-species variation (Fig. 2). PC1 of landmark displacements explained 67% of the variation within individuals (i.e., FA), 62% of the variation among individuals, and 70% of the variation among species (Fig. 2A–C). PC2 explained 21% of the variation within individuals, 25% of the variation among individuals, and 23% of the variation among species (Fig. 2D–F). PC3 explained 7% of the variation with individuals, 11% of the variation among individuals, and 6% of the variation among species (Fig. 2G–I).

For PC1, direction and magnitude of landmark displacements due to variation within individuals, among individuals, and among species were remarkably similar (Fig. 2A–C), whereas for both PC2 and PC3 the landmark displacement due to each effect were distinct (Fig. 2D–I). Across all PCs, coordinated displacements of landmarks occurred both within and outside of functionally integrated units. For example,

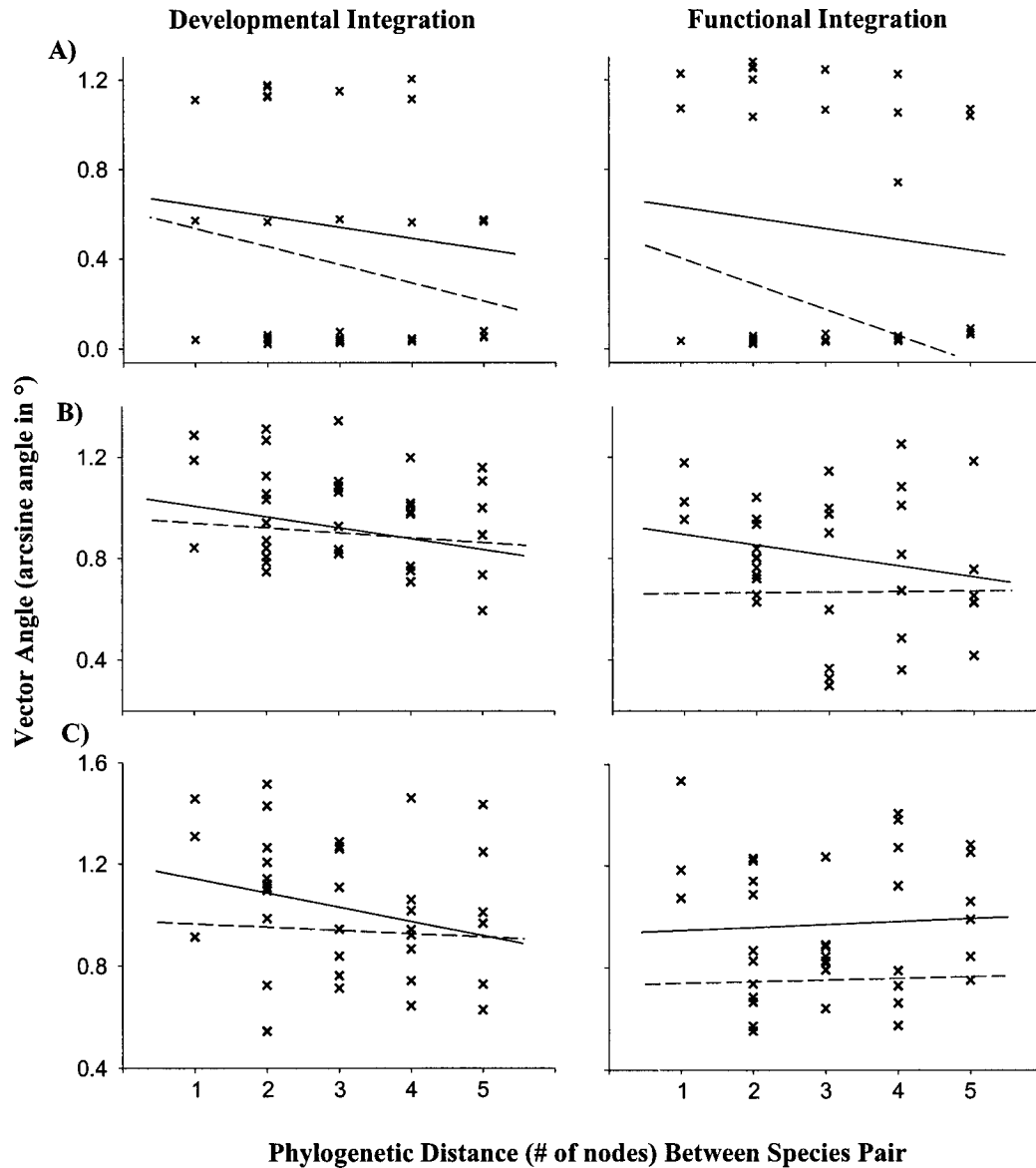


FIG. 4. The relationship between rank phylogenetic relatedness and arcsine-transformed vector angles for (A) PC1, (B) PC2, and (C) PC3 for developmental and functional integration for all species pair comparisons. Between-species vector angle reflects concordance of species pair integration pattern with larger vector angles indicating increased discordance of integration patterns. Solid lines indicate regression line for observed relationship between phylogenetic relatedness and morphological similarity. Dashed lines indicate regression line of relationship after removing the effects of phylogeny. For all graphs, observed and simulated data share the same slope indicating that more closely related species are not more similar in patterns of developmental or functional integration.

within functional units, PC1 displacements of landmarks 1 and 2, landmarks 3, 4, and 6, and landmarks 5 and 7 were similar in direction and magnitude for all effects (Fig. 2A–C). Outside of functional units, PC2 displacement of landmarks 12 and 13 were similar due to variation among species (Fig. 2F). While coordinated displacements of landmarks did occur both within and outside of functional units, comparisons of landmark displacements among individuals and species were more similar within than outside of functional units for all three PCs (Fig. 3; PC1: $t = -6.92$, $P < 0.01$; PC2: $t = -6.35$, $P < 0.01$; PC3: $t = -2.42$, $P < 0.05$). Likewise, comparisons of landmark displacements due to variation within and among individuals tended to be more similar with-

in than outside of functional units (Fig. 3; PC1: $t = 1.16$ $P = 0.2$; PC2: $t = 1.56$ $P = 0.1$; PC3: $t = 1.66$ $P = 0.1$).

Persistence of Developmental and Functional Integration

For PC1, intraspecific patterns of functional and developmental integration were similar among species (Table 1). Additionally, species pairs that were similar in patterns of developmental integration also shared patterns of functional integration (Table 1). For PC2 and PC3, among-species similarities of interspecific patterns of functional and developmental integration were lower than in PC1 (Table 1). Unlike PC1, among-species congruence of developmental integra-

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TABLE 1. Vector angles (in degrees) between principal components of landmark displacements within muscle attachment region (functional) and fluctuating asymmetry of all landmarks (developmental) for each *Sorex* species pair.

Species pairs		PC1		PC2		PC3	
		functional	developmental	functional	developmental	functional	developmental
<i>S. fumeus</i>	<i>S. haydeni</i>	1.9**	2.2**	59.3*	64.9*	48.7*	89.1
<i>S. fumeus</i>	<i>S. trowbridgii</i>	2.3**	1.9**	72.4	67.0*	60.4*	89.9
<i>S. fumeus</i>	<i>S. vagrans</i>	2.6**	4.3**	62.1*	63.5	55.6*	81.8
<i>S. haydeni</i>	<i>S. trowbridgii</i>	2.8**	2.3**	75.6	65.7*	69.9	58.9
<i>S. haydeni</i>	<i>S. vagrans</i>	2.8**	3.8**	81.9	87.7	64.0*	67.0
<i>S. trowbridgii</i>	<i>S. vagrans</i>	3.0**	3.8**	41.9*	76.6	48.8*	78.5
<i>S. cinereus</i>	<i>S. vagrans</i>	3.1**	3.0**	32.1*	72.0*	85.0	85.9
<i>S. fumeus</i>	<i>S. palustris</i>	3.1**	3.5**	50.6*	78.6	69.5*	80.5
<i>S. cinereus</i>	<i>S. haydeni</i>	3.2**	3.6**	73.4	83.5	79.1	89.4
<i>S. haydeni</i>	<i>S. palustris</i>	3.4**	3.0**	65.5	83.9	60.0*	54.1
<i>S. palustris</i>	<i>S. vagrans</i>	3.5**	3.6**	64.8	68.8*	56.9*	46.7
<i>S. cinereus</i>	<i>S. trowbridgii</i>	3.5**	4.4**	26.4*	79.3	65.6*	86.4
<i>S. palustris</i>	<i>S. trowbridgii</i>	3.9**	3.3**	88.9	74.5	88.8	60.9*
<i>S. cinereus</i>	<i>S. fumeus</i>	4.1**	4.4**	64.5*	61.1*	47.2*	80.0
<i>S. cinereus</i>	<i>S. palustris</i>	4.8**	3.8**	76.1	74.9	81.1	71.7*
<i>S. fumeus</i>	<i>S. pacificus</i>	5.0**	4.1**	85.4	62.6*	88.4	89.4
<i>S. pacificus</i>	<i>S. palustris</i>	5.1**	5.5**	73.4	85.9	81.8	59.7*
<i>S. haydeni</i>	<i>S. pacificus</i>	5.6**	5.0**	54.6*	60.3*	67.2*	74.1
<i>S. pacificus</i>	<i>S. vagrans</i>	5.9*	6.8*	74.4	79.6	67.2*	62.1
<i>S. pacificus</i>	<i>S. trowbridgii</i>	6.5*	4.7**	52.7*	50.3	61.4*	60.0
<i>S. cinereus</i>	<i>S. pacificus</i>	7.9*	7.1*	61.7*	75.7	85.5	89.2
<i>S. hoyi</i>	<i>S. monticolus</i>	60.8*	84.0	79.5	58.5*	55.3	76.5
<i>S. monticolus</i>	<i>S. palustris</i>	77.4*	48.2*	54.8	77.3	84.5	75.1
<i>S. haydeni</i>	<i>S. monticolus</i>	77.5*	48.9*	83.3	82.5	86.3	53.0*
<i>S. cinereus</i>	<i>S. monticolus</i>	77.6*	48.8*	36.4	70.1	78.5	76.3
<i>S. fumeus</i>	<i>S. monticolus</i>	78.3*	48.1*	56.1	76.1	63.8	72.8
<i>S. monticolus</i>	<i>S. vagrans</i>	78.8*	49.0*	28.9*	66.7	53.8	72.9
<i>S. monticolus</i>	<i>S. trowbridgii</i>	78.9*	48.3*	52.6	80.4	75.3	85.3
<i>S. monticolus</i>	<i>S. pacificus</i>	79.0*	48.6*	76.8*	67.1	83.3	86.9
<i>S. haydeni</i>	<i>S. hoyi</i>	83.9	81.2	66.9	72.7	84.8	84.1
<i>S. hoyi</i>	<i>S. pacificus</i>	84.7	80.8	31.7	61.4	86.0	68.6
<i>S. fumeus</i>	<i>S. hoyi</i>	84.7	80.6*	83.1	86.4	89.9	71.2
<i>S. hoyi</i>	<i>S. palustris</i>	85.3	82.1	70.5	80.4	66.1	85.7
<i>S. cinereus</i>	<i>S. hoyi</i>	85.4	82.8	60.2	87.0	66.2	85.8
<i>S. hoyi</i>	<i>S. trowbridgii</i>	85.6	81.3	52.8	81.3	79.7	81.0
<i>S. hoyi</i>	<i>S. vagrans</i>	86.2	83.1	77.7	78.3	68.6	80.4

* indicates significant difference from 90° and 0°, ** indicates significant difference from 90° but not 0°. All other values are significantly different from 0° but not 90°.

tion for PC2 and PC3 was not consistent with among-species similarity in functional integration (Table 1).

Species similarity in patterns of integration was not consistent with phylogenetic relatedness across all species pairs and PCs (Table 1; Figs. 1, 4). For both developmental and functional integration and for all PCs, the observed relationship between concordance of integration and phylogenetic relatedness among species was consistent with the simulated relationship controlling for phylogenetic relatedness (Fig. 4; test for homogeneity of slopes: PC1 developmental $P = 0.001$, functional $P = 0.03$; PC2 developmental $P = 0.01$, functional $P = 0.02$; PC3 developmental $P = 0.05$, functional $P = 0.05$).

DISCUSSION

Understanding the processes behind origin and evolution of complex morphological traits is an important goal of evolutionary biology (Lauder 1981; Raff 1996). While both developmental and functional requirements shape current morphology of complex traits, it is unclear whether these relationships bias trait evolution. Phenotypic patterns of devel-

opmental interactions among components of morphological structures are largely influenced by internal selection for developmental homeostasis, whereas patterns of functional integration are influenced by external natural selection (Schwenk and Wagner 2001). Thus, concordant patterns of developmental and functional integration result from consistency of internal or external selection across environments, and evolutionary persistence of patterns of integration can reflect the relative importance of these two processes for origin and evolution of morphological integration (e.g., Caumul and Polly 2005).

We identified contemporary patterns of developmental and functional integration in mandibles of nine species of soricid shrews, examined similarity of integration patterns across species, and characterized historical persistence of developmental and functional integration by comparing observed interspecific integration patterns with those expected based on the historical relationships among taxa. We documented highly concordant patterns of developmental and functional integration across most species, and a strong consistency of among-species concordance of developmental and functional

integration (Table 1). Whereas among-species concordance of integration was high for the majority of species pairs, similarity in developmental and functional integration was independent of species relatedness (Table 1; Figs. 1, 4). Moreover, direction and magnitude of mandible shape variation across individuals and species was more similar within than outside of functional units (Figs. 2, 3).

Close among-species concordance in patterns of developmental and functional integration (Table 1) suggests that the same developmental pathways channel both functional and developmental variation (Cheverud 1982; Badyaev and Foresman 2004). Such channeling can result in similarity of developmental and functional integration when trait development is highly sensitive to the environment, allowing mandible function to direct mandible development. For example, muscle loading often affects initiation of ossification in bone structures (Herring and Lakars 1981; Herring 1993; Hiiemae 2000). In *Sorex*, high environmental sensitivity of mandible development is enabled by delayed ossification of the mandible that coincides with onset of independent foraging (Foresman 1994). By channeling developmental and functional variation, shared developmental pathways can determine the variation available for selection. Thus, if the same developmental pathways channel developmental and functional variation in shrews, then variation among individuals and species should be similar to variation produced during development (i.e., within-individual variation). Indeed, we found that within-individual, among-individual, and among-species variation in mandible shape were remarkably similar (similar direction and magnitude of landmark displacements; Fig. 2A–C). Moreover, the high level of similarity in interspecific developmental and functional integration patterns may result from similar channeling of developmental and functional variation (similar vector angles for developmental and functional comparisons; Table 1, PC1).

Among-species similarity in channeling of developmental and functional variation indicates consistent selection for developmental homeostasis across species. Interestingly, despite this similarity of selection across species, congruence of integration patterns did not vary with their phylogenetic relatedness (Table 1, Figs. 1, 4). Such strong concordance in integration patterns among study species accompanied by lack of phylogenetic dependence suggests that similarity in morphology resulted from shared aspects of development and function rather than shared evolutionary histories. For example, species may be highly similar in muscle attachment and thus location of mechanical load, which in turn influences ossification, bone formation, and accumulation of phenotypic variation (Herring 1993; Zelditch 2005). However, magnitude and direction of such mechanical load depends on the environment (e.g., diet), thereby producing greater convergence of species with similar ecological requirements. Indeed, in a concurrent study, we found that similarity in mandible traits reflect similarity in diet preferences regardless of phylogenetic relatedness (R. L. Young and A. V. Badyaev, unpubl. ms.). For example, *S. hoyi* and *S. monticolus* were distinct from other study species in patterns of developmental and functional integration (Table 1), suggesting that muscle attachment regions in these species have diverged from other *Sorex*. However, this seems unlikely as dissections of three

distantly related species of *Sorex* (including *S. monticolus*) revealed highly conserved muscle attachment locations (Badyaev et al. 2005). Instead, this discordance is likely produced by distinct ecological requirements experienced by these two species; while most *Sorex* are generalist predators, *S. monticolus* specializes on soft-bodied prey (Carraway and Verts 1994). Alternatively, lack of phylogenetic dependence could result from incorrect assignment of phylogenetic relatedness among species. However, these patterns of phylogenetic relatedness are highly supported (Fumagalli et al. 1999; see concordant findings in Demboski and Cook 2001, 2003; Ohdachi et al. 2001), and are likely robust to minor changes in assigned phylogenetic relatedness because well-supported sister taxa (e.g., Demboski and Cook 2001, 2003) are as divergent in patterns of developmental and functional integration as much more distantly related taxa (Fig. 4).

Strong environment-independent selection for developmental homeostasis may channel the accumulation of developmental and functional variation, thus determining patterns of morphological integration. At the same time, both consistency of integration patterns within functional units across levels (i.e., within individuals, among individuals, and among species) and the weak relationship between patterns of integration and species relatedness suggests that species differences in functional demands during mandible development results in observed divergence of mandibular morphology. Further examination of species variation of mandible function (e.g., bite force and prey capture) and development (e.g., ontogeny of muscle loading) is necessary to elucidate the proximate mechanism underlying these patterns. Overall, our results emphasize the importance of considering the source of integration for inferring evolutionary change in complex morphological structures.

ACKNOWLEDGMENTS

We thank the Museum of Southwestern Biology (UNM, Albuquerque) and W. L. Gannon, the Museum of Vertebrate Zoology (UC, Berkeley), and the U.S. National Museum of Natural History for providing access to their *Sorex* collections; K. R. Foresman for help with shrew collection in Montana; C. P. Klingenberg for providing the SAS IML program for Procrustes superimposition; and M. D. Dean for providing the Python program for data simulations. We thank J. B. Walsh, M. Worobey, K. P. Oh, D. Acevedo Seaman, R. A. Duckworth, P. Edelaar, and two anonymous reviewers for discussions and comments that improved this manuscript and the National Science Foundation (IOB-0447534) and the University of Arizona for partially funding this study.

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Corresponding Editor: E. Brainerd