RESEARCH ARTICLE

Developmental Plasticity Links Local Adaptation and Evolutionary Diversification in Foraging Morphology

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ABSTRACT

Developmental plasticity is thought to reconcile the constraining role of natural selection in maintaining local adaptation with evolutionary diversification under novel conditions, but empirical documentations are rare. In vertebrates, growth and development of bones is partially guided by contractions of attached musculature and such muscle activity changes progressively through embryonic development from sporadic motility to direct functional effects. In species with short generation times, delayed skull maturation extends the guiding effects of muscle activity on formation of foraging morphology into adulthood, providing an opportunity to directly examine the links between plasticity of bone development, ecological adaptations, and evolutionary diversification in foraging morphology. In this case, the morphological consequences of inputs due to local functional requirements should be evident in adaptive divergence across taxa. Here we provide evidence that epigenetic regulation of bone growth in Soricid shrews may enable both development of local adaptations and evolutionary divergence in mandibular morphology. We contrast the effects of muscle stimulation on early- vs. late-maturing components of, foraging apparatus to show that the morphology of late-maturing components is more affected by functional requirements than are early-ossifying traits. Further, the divergence in foraging morphology across shrew species occurs along the directions delineated by inductive effects of muscle loading and bite force on bone formation in late-maturing but not early-maturing mandible components within species. These results support the hypothesis that developmental plasticity can link maintenance of local adaptations with evolutionary diversification in morphology. *J. Exp. Zool. (Mol. Dev. Evol.) 314B:434–444, 2010.* © 2010 Wiley-Liss, Inc.

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Differences in resource availability across environments and competition within an environment favor the evolution of morphological diversity (Simpson, '44; Brown and Wilson, '56; Van Valen, '65; Losos, 2000; Schluter, 2000), and such diversification depends crucially on the presence of developmental variation (Schmalhausen, '38; Waddington, '41; West-Eberhard, 2003). On the one hand, greater developmental plasticity enables exploitation of diverse ecological resources and can thus fuel extensive adaptive radiations (Baldwin, '02; West-Eberhard, '89). On the other hand, selection for precise local adaptation hinders further developmental innovations enforcing stasis of adapted forms (Lewontin, '83; Vermeij, '96). Developmental plasticity is thought to link local adaptation and evolutionary diversifications—thus providing continuity in evolutionary

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processes (Schmalhausen, '38; West-Eberhard, 2003); however, empirical documentations are rare.

Epigenetic regulation of skeletal development by attached musculature provides an opportunity to explore evolutionary consequences of developmental plasticity. Because the causes of muscle activity vary through ontogeny, their effects on skeletal formation directly link developmental plasticity and current functional requirements of the local environment. For example, early in ontogeny, bone growth and maturation is influenced by internal factors, such as embryonic motility (Müller, 2003), that establish coordinated development of major skeletal components and initial integration of soft and hard skeletal tissues (Bertram and Swartz, '91; Enlow, 2000). Late in ontogeny, when muscle actions are guided by functional demands from the environment, their effects on bone remodeling and skeletal morphology are directly linked to locally adaptive functions (Frost, '87). Thus, the role of muscle–bone interactions in the development of adaptations depends on the timing of bone formation in relation to the onset of functional use (Young and Badyaev, 2007).

Particularly instructive in this respect are mammalian species in which delayed ossification of the foraging apparatus extends the effects of muscle activity on growth and development of mandibular morphology into postweaning independent foraging. This delayed ossification enables examination of the directing role of ecological variation on bone development (e.g., Badyaev and Foresman, 2000; Badyaev et al., 2005). In soricid shrews (*Sorex* sp.), mandible growth and development occurs late in ontogeny, coinciding temporally with independent foraging (Vogel, '73; Foresman, '94; Masuda and Yohro, '94; Badyaev and Foresman, 2004). This delayed ossification of the mandible allows ecologically relevant muscle activity to direct mandible development such that ecology is a primary source of developmental variation in mandible morphology (Young and Badyaev, 2006). Here, we show that the development of late-maturing mandibular components is influenced by local functional demands of foraging and test the hypothesis that this plasticity in mandible development can link morphological adaptation and diversification within and across shrew species. Specifically, we contrast the effects of foraging-linked muscle activity on development of early- and late-ossifying mandibular components in a species of Sorex shrews and compare their contribution to evolutionary divergence in mandibular morphology among related species. We suggest that variation in late-ossifying regions associated with muscle use due to foraging is critical for local adaptation in mandibular morphology within a species and further, contributes more to morphological divergence across species.

MATERIAL AND METHODS

Mechanics and Musculature of the Shrew Mandible

Foraging in shrews is enabled by jaw movements associated with prey capture (jaw opening), initial crushing of prey, and prey processing (positioning, shearing, and grinding of prey) (Dötsch, '82, '94). Several muscles contribute to jaw movement (reviewed in Sharma, '58); however, over 90% masticatory function is produced by action of three muscles: *Musculus temporalis*, *M. masseter*, and *M. digastricus* (Dötsch, '85, '94). The *M. temporalis* and *M. masseter* attach to the late-ossifying region of the mandible (Fig. 1B) and are the primary masticatory

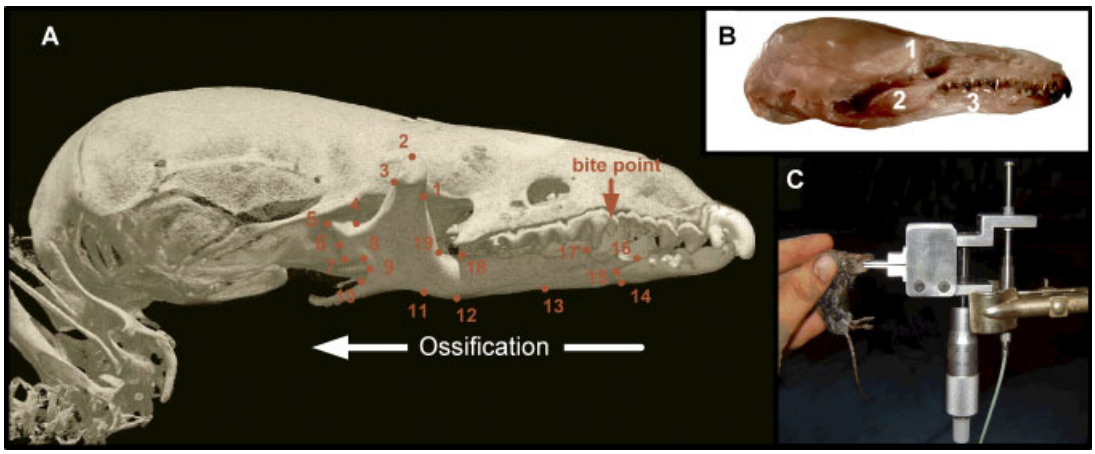

Figure 1. Characteristics of development and assessment of form and function of shrew foraging morphology. (**A**) Shrew mandible ossification starts in the anterior, tooth-bearing, region and proceeds posteriorly toward the articulation with the skull. The early-ossifying region is delineated by landmarks 11–19, and the late-ossifying region by landmarks 1–10. The articulation point of the mandible with the skull, the condyle, is outlined by landmarks 5–7. The arrow indicates the major bite point of the jaw. (**B**) Attachment locations of muscles *M. temporalis* (1), *M. masseter* (2), and *M. digastricus* (3) on the shrew mandible (for expanded functional description see text). (**C**) Bite force of live-captured *Sorex monticolus* was measured with Kistler force transducer and charge amplifier.

muscles exerting the prey crushing and grinding forces. The *M. digastricus* attaches to the early-ossifying region of the mandible (Fig. 1B), is the primary muscle of jaw opening (Dötsch, '85, '94), and is an important determinant of gape angle (Carraway and Verts, '94; Young et al., 2007). Owing to its role in determining gape angle, function of the *M. digastricus* is a critical determinant of the maximum biting force of the foraging apparatus (Carraway and Verts, '94; Young et al., 2007). Contraction of muscles generates force directly on the region of attachment and indirectly on other functional components of the mandible (e.g., the joint and dentition). First, contraction of all muscles—*M. temporalis*, *M. masseter*, and *M. digastricus*—results in movement of the jaw and generates force on the mandibular articulation, through the force of the joint against the skull articulation, resulting in mechanical stimulation on the mandible joint (the condyle, Fig. 1A; Herrel et al., '98b; Hiiemae, 2000). Second, because capture and mastication of prey items exerts force on the dentition (Dötsch, '85; Herrel et al., '98b), action of the *M. temporalis* and *M. masseter* associated with crushing, handling, and grinding of prey items results in mechanical stimulation of associated mandibular region. These inputs are proportional to the action of the muscles, and we measured foraging-linked inputs of these muscles and their associated influences on skeletal morphology of the mandible.

Data Collection

Bite Force Performance. In accordance with standards of animal care and use (IACUC ♯04–090), we captured 26 montane shrews (*Sorex monticolus*) in the Pinaleño and Jemez mountain ranges of southeastern Arizona and north central New Mexico in June–August 2005. Upon capture, we measured in vivo bite force using bite plates attached to type 9203 Kistler force transducer and type 5995 charge amplifier (Kistler Inc., Winterthur, Switzerland) (after Herrel et al., 2001). Bite force was measured three times for each individual and the highest value was recorded. To standardize measurements across individuals, bite plates were opened by 1 mm between all repeated measures and all individuals.

Estimation of Muscle Loading. After bite force measurements, individuals were sacrificed and fixed in 10% buffered formalin and muscle location, orientation, mass, and average fiber length were measured for *M. digastricus*, *M. masseter*, and *M. temporalis* (Fig. 1B). Muscle orientation, location of attachment, and three dimension coordinates of origin on the cranium and insertion on the mandible were measured in relation to the articulation point of the mandible and cranium (the condyle; Fig. 1A, landmarks 5–7). Muscles were dissected off the mandible, weighed (with 0.01 mg resolution) using a Mettler Toledo AB135-S/FACT balance (Columbus, OH), immersed in 40% nitric acid (HNO_3) until muscle fibers separated (24–30 hr), and stored in a 50% aqueous glycerol solution (Herrel et al., '98a). Muscle fibers were photographed under 10–12.5 × magnifications with Leica DC 300 microscope (Bannockburn, IL), sizes were standardized using a ruler photographed along with the fibers. Fiber length of each muscle was the mean of measurements of 20 different fibers. Force of each muscle was calculated as the product of the physiological cross-section (cm^2) and a force conversion factor ($C = 25$) (Herzog, '94), where physiological cross-section was the quotient of muscle mass (g) and mean fiber length (cm) (Herrel et al., '98b). We estimated muscle loading, or exertion of each muscle on the mandible, as the force distributed across the area of attachment. Area was measured as the ellipse formed by the length—the longest measure of muscle attachment—and the height—the perpendicular measure—of the muscle attachment. We examined contribution of each of the three muscles to bite force performance by regressing bite force on estimated values of muscle loading (Fig. 2).

Skull Morphology. Left and right mandibles were separated, placed on a slide, and photographed under 10 × magnification using a Leica DC 300. We standardized size using a ruler photographed with the mandibles. To assess mandible shape, *x*- and *y*-coordinates of 19 landmarks distributed across the mandible were obtained from mandible images (Fig. 1A). Each individual and side was measured twice to assess measurement error. All data collection from images was done using tpsDig2 (Rohlf, 2006). The mandible was divided into early- and late-ossifying regions (Fig. 1A) based on a priori knowledge of developmental sequence and timing of ossification in the mandible (Vogel, '73; Yamada and Yohro, '88; Masuda and Yohro, '94; Foresman, '94). We estimated size of each mandibular region as centroid size—the square root of the summed squared distance of each landmark to the center of the region.

Skull Histology. To assess differences in postossification bone remodeling between the early- and late-ossifying mandibular regions, formalin-fixed mandibles were decalcified and sectioned sagitally (5 μm, Fig. 5A). Sections were stained with hematoxylin and eosin and photographed under 100 × magnification using a QImaging MicroPublisher 3.3 RTV attached to a Nikon Eclipse TE2000-U inverted microscope (Fig. 5A). We measured two indices of bone remodeling (after Hedgecock et al., 2007): density of secondary osteons (Fig. 5B) and density of osteocytes (Fig. 5C). We compared density of secondary osteons and osteocytes between the early- and late-ossifying regions of the mandible using a Wilcoxon two-sample test. Secondary osteons develop from the remodeling of existing bone (Young et al., 2006) and increase in density under induced bone remodeling of the mandible (i.e., presence of cementing lines, Tran Van et al., '82), and osteocytes receive mechanical inputs and initiate bone remodeling (Noble et al., 2003). As a result, density of these two factors can be used as an index of the degree of bone remodeling (Hedgecock et al., 2007). Secondary osteons were identified by the presence of a cementing line marking the boundary of bone resorption and osteocytes were identified by cell morphology.

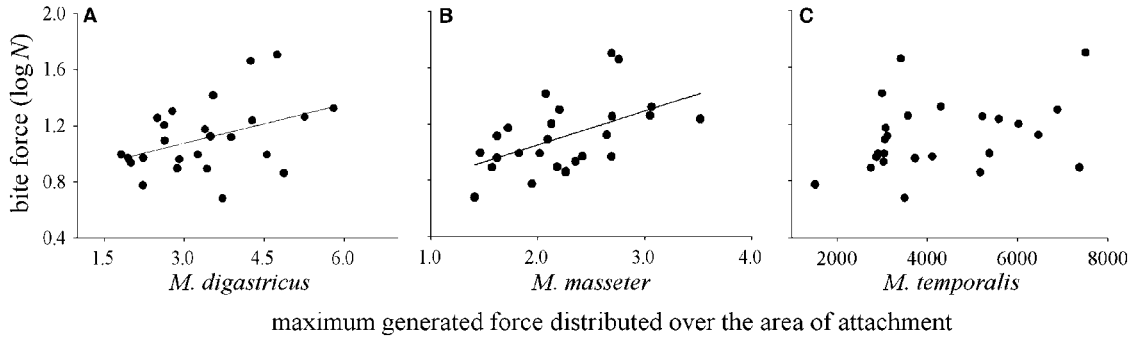

Figure 2. Contribution of muscle loading to empirically measured bite force. Function of muscles *M. digastricus* and *M. masseter* influenced individual bite force (A) *M. digastricus:* $b_{ST} = 0.41$, $P < 0.05$; (B) *M. masseter:* $b_{ST} = 0.53$, $P < 0.01$. (C) Although higher maximum force of the *M. temporalis* tended to result in increased bite force ($b_{ST} = 0.34$, $P = 0.09$), this association was not significant, and may reflect the additional role of the *M. temporalis* for lateral movement of the left and right mandibles characteristic of shrew mandible function (Dötsch, '85, '94). Muscle loading was estimated as the maximum force generated by each muscle distributed over the area of muscle attachment. Maximum force of each muscle was estimated as (muscle mass/mean fiber length) × force conversion factor.

Data Analysis

Mandible Size, Shape, Bite Force, and Developmental Variation. To separate variation due to mandible size and shape, 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, individuals, body sides, and repeated measures using a single Procrustes superimposition (generalized orthogonal least-squares fit, Rohlf and Slice, '90). A principal component (PC) analysis of landmark configurations (Procrustes coordinates) was used to summarize the major axes of mandible shape variation for early- and late-ossifying regions independently. To examine the relationship between bite force and mandible form, we regressed bite force on centroid size and the first three PCs of shape for both regions of the mandible. Regression analyses were done before and after Procrustes shape coordinates were corrected for allometric variation. We corrected for allometric variation in shape using a multiple regression of the Procrustes shape coordinates on centroid size of the early- and late-maturing regions of the mandible independently.

We assessed developmental variation in mandible shape by measuring the covariation of developmental errors (fluctuating asymmetry (FA)) between the left and right sides of the mandible for all 19 landmarks (after Klingenberg et al., 2001; Klingenberg, 2003). Because trait variation due to FA results from random perturbations during development (reviewed in Hallgrímsson, '99), FA variation is assumed to be randomly distributed unless traits share direct developmental links. As a result, concordance in direction and magnitude of FA covariation and mandible variation due to other sources (e.g., mandible function or muscle loading) indicate that accumulation of variation due to these independent sources is regulated by the same developmental processes (e.g., Badyaev and Foresman, 2000; Klingenberg, 2003; Young and Badyaev, 2006).

To examine differences in mandible shape variation due to distinct effects, we partitioned variation in landmark configurations among individuals, among sides, due to developmental variation (individual by side interaction), and due to measurement error (repeated measures) using a Procrustes analysis of variance (ANOVA) (Table 1; Goodall, '91; Klingenberg and McIntyre, '98; Badyaev and Foresman, 2000) including estimated muscle loadings as covariates (Zelditch et al., 2004), where individual identity and repeated measure were entered as a random effects. We evaluated the early- and late-ossifying regions separately by including only landmarks within each region in the analysis (Fig. 1). To examine differences in the development of variation in the early- and late-maturing regions of the mandible, we assessed morphological variation among individuals and due to accumulation of fluctuating asymmetries (i.e., errors in normal development) in early- and late-ossifying regions of the mandible. Shape variance among individuals and due to developmental errors (individual by side interaction) was estimated as the sum of the eigenvalues of the covariance matrices (or the sum of the univariate variances) calculated based on the expected mean squares matrices of sums of squares and cross products for early- and late-maturing regions separately.

Effects of Muscle Loading on Developmental and Individual Variation in Mandible Shape. To examine the relationship between muscle loading and individual and developmental (assessment methods described above) variation in mandible shape, we examined the covariation between landmark displacements due to each effect and loading of each muscle using partial least squares (PLS) analysis. Specifically, the covariation between

Table 1. Partitioning of mandible shape variation among individuals, sides, FA, and measurement error using Procrustes ANOVA.

	Early-ossifying region			Late-ossifying region		
Effect	df	MS	*F*	df	MS	*F*
Individual	336	0.0017	4.39**	384	0.0015	3.06**
Side	14	0.0048	12.0**	16	0.0025	5.15**
Individual × side (FA)	336	0.0004	3.66**	384	0.0005	2.57*
Measurement error	14	0.0001	–	16	0.0002	–

The *F*-values of individual and side were calculated with the MS of FA as the denominator, and the FA *F*-value was calculated with MS of measurement error as the denominator.
*$P<0.05$, **$P<0.01$.

Table 2. Covariation of mandible shape variation—among individuals and due to developmental errors —and estimated muscle loading using partial least squares analysis.

	Early ossifying				Late ossifying			
	Individual		Developmental errors		Individual		Developmental errors	
Muscle	SV	*r*	SV	*r*	SV	*r*	SV	*r*
M. digastricus	0.003	0.68	0.046	0.54	0.004**	0.72	0.0029*	0.75
M. masseter	0.0029*	0.69	0.036	0.60	0.003**	0.73	0.0023**	0.77
M. temporalis	0.0126	0.70	0.15	0.41	0.0015*	0.66	0.0121	0.72

The PLS singular value (SV) and correlation (*r*) of each muscle-shape variation comparison are provided.
*$P<0.01$, **$P<0.05$.

mandible shape variation and estimated loading of each muscle was calculated using PLS analysis of Procrustes shape coordinates and estimated loading of each muscle (after Adams and Rohlf, 2000). After partitioning shape variation among effects, the covariation between developmental variation in shape (individual by side interaction) and estimated loading of each muscle was calculated using PLS analyses (as above). Significance of the PLS singular value of each comparison was tested using a permutation test (Table 2) (after Rohlf and Corti, 2000). To compare directionality of muscle-related developmental variation and muscle-related individual variation in mandible shape, we calculated the angle between the single PLS axis describing the directionality of each muscle-shape comparison as: $\Theta = \arccos[\mathbf{a} \cdot \mathbf{b}/(|\mathbf{a}| \cdot |\mathbf{b}|)]$, where a and b are vectors of each shape coordinate, and |a| and |b| are their lengths (Zelditch et al., 2004). A more acute angle indicates greater similarity. PLS analyses and vector angle calculations (means ± bootstrapped s.d.) were performed on the early- and late-maturing regions of the mandible independently. Bootstrapped standard deviations were calculated by sampling with replacement *x*- and *y*-coordinates ($n = 1{,}000$ iterations), recalculating the PLS axes of each effect, and recalculating the vector angles between each pair of effects. The effect of muscle loading on mandible size was measured with a multiple regression with centroid size as a dependent variable and our estimate of muscle loading (for the *M. digastricus*, *M. masseter*, and *M. temporalis*) as the independent variables.

Interspecific Diversity in Mandible Morphology. To assess the importance of timing of development for morphological divergence among taxa, mandible size and shape variation were partitioned using a single Procrustes superimposition of nine species of *Sorex* shrews: *S. cinereus, S. fumeus, S. haydeni, S. hoyi, S. monticolus, S. pacificus, S. palustris, S. trowbridgii, and S. vagrans* (for more details about this sample see Young and Badyaev, 2006). Mean centroid size was calculated for each species and each region of the mandible. We compared interspecific variation in size of early- and late-ossifying regions of the mandible with Levene's test (Schultz, '85). Variation in mandible shape was partitioned among effects of species, individual identity, developmental variation and measurement error using Procrustes ANOVA (Table 3; Goodall, '91; Badyaev and Foresman, 2000), where individual identity was nested within species and entered as a random effect and repeated measure was entered as a random effect. After partitioning, shape variance among species was estimated as the sum of the eigenvalues of the covariance matrices calculated based on the expected mean squares matrices of sums of squares and cross

Table 3. Partitioning of mandible shape variation among species, individuals, sides, FA, and measurement error using Procrustes ANOVA.

	Early-ossifying region			Late-ossifying region		
Effect	df	MS	*F*	df	MS	*F*
Species	112	0.6222	7.33**	128	0.4838	7.25**
Individual	2198	0.1531	1.80*	2512	0.1028	1.54**
Side	126	0.9195	10.8**	144	0.7124	10.7**
Individual × side (FA)	2058	0.0848	3.88**	2352	0.0668	8.82**
Measurement error	14	0.0021	–	16	0.0078	–

The *F*-values of species, individual, and side were calculated with the MS of FA as the denominator, and the FA *F*-value was calculated with MS of measurement error as the denominator.
*$P<0.05$, **$P<0.01$.

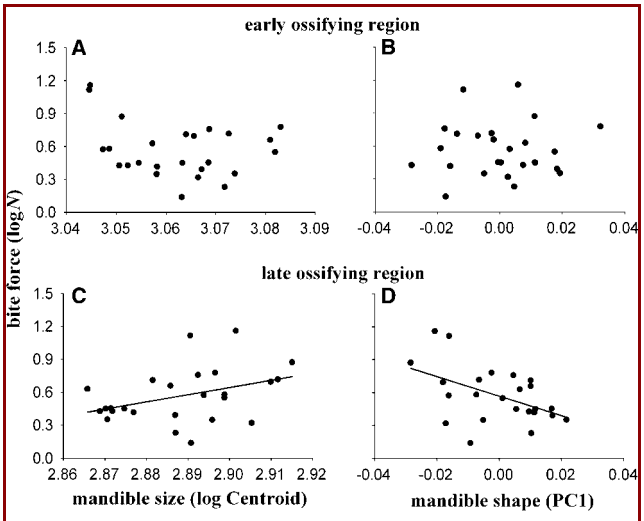

Figure 3. Contribution of size and shape of the early- and late-ossifying regions of the mandible to empirically measured bite force. Neither variation in (A) size, $b_{ST} = -0.27$, $P = 0.2$, nor (B) shape, $b_{ST} = -0.12$, $P = 0.6$, of the early-ossifying region contributed to bite force. In the late-ossifying region, variation in (C) size, $b_{ST} = 0.39$, $P < 0.06$, and (D) shape, $b_{ST} = -0.58$, $P < 0.01$, contributed to bite force. The lines show regression of bite force on size (measured as the centroid size of landmarks contained in the early- and late-maturing regions separately) and shape (measured as the first principal component of shape describing 34.8% of variation in landmarks contained in the early-ossifying region and 33.9% of variation in landmarks contained in the late-ossifying region). Regressions of bite force on additional shape PCs are provided in Table 4.

products for early- and late-maturing regions separately (as above). Shape variances, generated from Procrustes superimposed shape variables, were rescaled to adjust for size differences between the regions by multiplying the multivariate variance by the log mean centroid size for the appropriate region. To compare directionality of muscle-related variation and interspecific divergence in shape of the early- and late-ossifying regions of the mandible, we assessed concordance of landmark displacements associated with shape divergence across species and shape variation within species associated with estimated muscle loading. We assessed interspecific divergence in shape using canonical variates analysis (CVA; Zelditch et al., 2004). We calculated the angle (mean ± bootstrapped s.d.) between the first CVA axis of species divergence and the single PLS axis describing shape variation associated with muscle loading in the early- and late-ossifying regions separately.

RESULTS AND DISCUSSION

Muscle Activity and Ossification in the Mandible

Both early- and late-maturing regions of the shrew mandible (Fig. 1) are exposed to muscle loading associated with foraging (Fig. 2); however, because the two mandible regions experience these inputs at distinct developmental stages, the consequences of muscle activity differ. We first compared the effects of muscle loading on mandible size and shape—two traits important for generating bite force (Fig. 3; Table 4) —in the early- and late-ossifying regions of the mandible in *S. monticolus*. Muscle loading associated with biting (Fig. 2) strongly influenced development of mandibular size and shape in the late, but not the early-ossifying region (Table 5; size: early ossifying $-F = 0.04$, $P = 0.99$, late ossifying—$F = 2.68$, $P = 0.05$; Fig. 4A and B). The morphological effects of muscle loading on the late-ossifying region resulted primarily from changes in size (Table 5) and size-related shape (Fig. 4B); however, modifications of shape unrelated to size were also evident (Fig. 4A). Adjustments in size in response to foraging-related muscle loading (Fig. 2) corroborates previous findings of the close relationship between size of the late-maturing region of the mandible and bite force across taxa (Young et al., 2007).

The distinct effects of muscle activity in the early- vs. late-ossifying regions of the mandible influenced developmental

Table 4. Shape of the late, but not early-ossifying region of the mandible, contributed to bite force.

	Early ossifying		Late ossifying	
Principal component	% variation	b_{ST}	% variation	b_{ST}
PC1	34.8	−0.12	33.9	−0.58**
PC2	26.6	0.31	19.3	−0.41*
PC3	10.6	0.27	13.7	−0.26
$PC1^{allometry}$	31.7	0.009	26.7	−0.40*
$PC2^{allometry}$	23.2	−0.31	18.7	−0.20
$PC3^{allometry}$	12.2	−0.23	14.5	−0.16

Shown are the regressions of bite force on the first three PCs of mandible shape variation and the first three PCs of shape after correcting for allometric variation ($PC^{allometry}$) in the early- and late-ossifying region of the mandible. b_{ST} is the standardized regression coefficient.
*$P<0.05$, **$P<0.01$.

Table 5. Effect of muscle loading on size of early- and late-ossifying mandibular regions.

	M. digastricus		*M. masseter*		*M. temporalis*	
Ossification timing	b_{ST}	t	b_{ST}	t	b_{ST}	t
Early	0.01	−0.06	0.07	−0.3	0.01	0.06
Late	0.41**	2.04**	−0.44**	−2.3**	0.18	−0.9

Force of muscle influenced size in late, but not early-ossifying mandibular regions of muscle attachment. Muscles producing bite force attach to both regions of the mandible (Fig. 1), but the muscle effects are limited to the late ossifying region only. Shown are multiple regressions of mandible size on estimated muscle activity. b_{ST} is the standardized partial regression coefficient.
*$P<0.05$, **$P<0.01$.

variation in the two regions. The early- and late-maturing regions of the mandible had similar levels of variation among individuals, but the late-maturing region showed lower levels of FA (i.e., accumulation of random errors during development of the left and right sides of the mandible; Fig. 4D). Differences in FA between the early- and late-ossifying regions of the mandible are indicative of differences in ontogenetic accumulation of developmental errors in the two mandibular regions (reviewed in Hallgrímsson, '99). Specifically, reduced FA in the late-maturing region suggests that foraging-linked muscle activity may direct development of this region and minimize the accumulation of random developmental errors (Badyaev and Foresman, 2000; Badyaev et al., 2005). In fact, in a previous study examining the distribution of developmental errors across the shrew mandible in highly stressful environments, high levels of FA were limited to areas outside of muscle attachment regions corroborating the role of muscles in directing the developmental accumulation of FA (Badyaev et al., 2005). Together this evidence provides additional support for the hypothesis that development and ossification of these two regions are regulated by distinct internal and external functional demands. Importantly, our results suggest that the muscle loading that induced differences between early- and late-ossifying regions of the mandible resulted from adjustments of bone development (Fig. 4) rather than postossification remodeling; histological evidence suggested similar levels of bone remodeling—estimated as secondary osteon and osteocyte density—in the early- and late-maturing regions of the mandible (Fig. 5). These results suggest that muscle activity has particularly strong effect on development of the late-ossifying region of the mandible and implicate ontogenetic timing of bone formation in determining the morphological effects of muscle activity. Alternatively, because evidence of remodeling is present in both the early- and late-ossifying regions of the mandible, the two regions may respond to different environmental stimuli resulting in an association between foraging-related muscle activity and morphology in the late, but not early-ossifying region of the mandible. However, forces associated with foraging should impact both the early- and late-maturing regions of the mandible through the direct and indirect effects of muscle loading and prey processing (see discussion of mandible mechanics above). A more thorough investigation of the relationship between, ossification timing, foraging, and mandible remodeling is required to fully distinguish between these alternative interpretations.

Mandibular Morphology and Bite Force

The functional demands on mandibular form vary widely across environments and diets in shrews (Young et al., 2007).

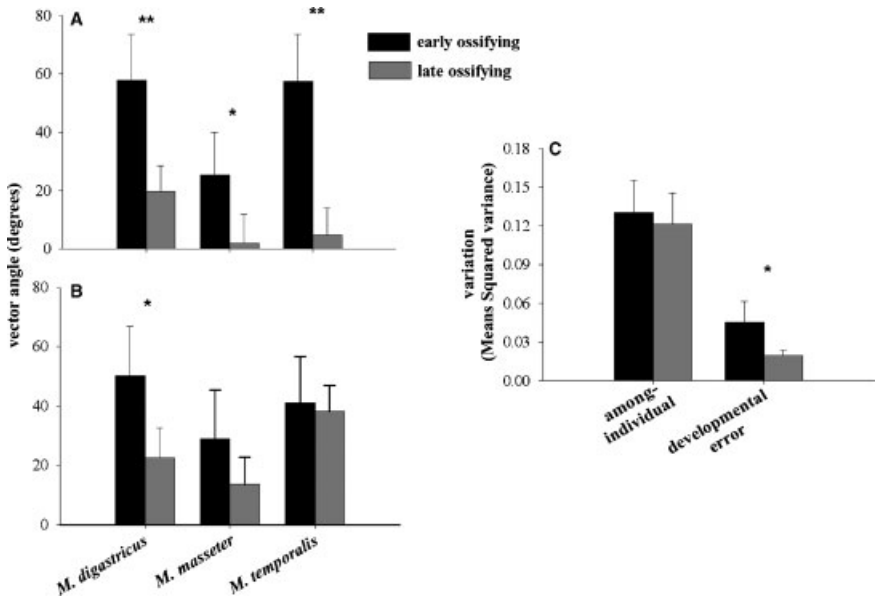

Figure 4. Muscle loading associated with foraging influences development and morphology in the late-ossifying region of the mandible. Both direct developmental effects of muscle loading on the region of attachment and indirect effects of muscle loading outside of the attachment region were observed (see description of mechanics and musculature in methods). However, in general, individual variation in shape associated with muscle loading was more concordant with muscle-related developmental variation in the late-ossifying region. Concordance in patterns of covariation is indicated by the angle between the single PLS axis describing the directionality of developmental variation associated with loading each muscle (*M. digastricus*, *M. masseter*, and *M. temporalis*) and the PLS axis describing directionality of mandible shape variation among individuals associated with loading of each muscle before (**A**) and after (**B**) correcting for allometric variation in mandible shape. Shown are angles (mean $\pm$ bootstrapped s.d.) between vectors of developmental variation and mandible shape variation associated with loading of each muscle. More acute angles indicate greater developmental effect of muscle loading. Developmental variation is measured as covariation among landmarks in fluctuating asymmetry (see *methods*). (A) Before correcting for allometric variation in shape, concordance between individual variation in shape associated with muscle loading and developmental variation associated with muscle loading was significantly higher in the late-ossifying region of the mandible (*M. digastricus;* $t = 4.6$, $P < 0.01$; *M. masseter*: $t = 2.9$, $P < 0.05$; *M. temporalis:* $t = 6.1$, $P < 0.01$). (B) After correcting for allometric variation in shape, *M. digastricus*-related individual variation and *M. digastricus*-related developmental variation were highly concordant in the late, but not the early-ossifying region of the mandible ($t = 2.8$, $P < 0.03$). The developmental effects of *M. masseter* tended to be more concordant with *M. masseter*-related individual variation in mandible shape in the late-ossifying region of the mandible; however, this relationship was not significant ($t = 1.9$, $P = 0.09$). There was no difference between the early- and late-ossifying region in concordance of developmental and individual variation associated with loading of the *M. temporalis* ($t = 0.3$, $P = 0.8$). (**C**) Developmental accumulation of variation differed between the early- and late-ossifying regions. The magnitude of shape variation among individuals within the population (measured as the means square variance of shape among individuals) did not differ between the early- and late-ossifying regions (variance: early = 0.13, late = 0.12, $t = 1.07$, $P = 0.3$). However, the accumulation of random developmental errors, measured as the magnitude of FA, was lower in the late-ossifying region (variance: early = 0.05, late = 0.02, $t = 6.98$, $P < 0.01$). One asterisk indicates $P < 0.05$ and two asterisks indicated $P < 0.01$.

We compared the contribution of mandibular morphology to bite force in *S. monticolus* (Fig. 1C) and found that size and shape of the late, but not early-ossifying region of the mandible strongly contributed to bite force (Fig. 3; Table 5). Because muscle loading was strongly associated with bite force (Fig. 2), differences in contribution of the early-and late-maturing regions of the mandible to bite force likely reflect differences in the role of muscle action in regulating bone growth and development of the two regions (Table 5; Figs. 4 and 5). Specifically, the findings reveal that morphology of the late-ossifying mandibular region was adjusted to meet the functional requirements of bite force, and suggests that delayed development of the late-ossifying region may enable the plastic development of locally adaptive morphologies across environments and taxa (e.g., Hall '84, Meyer, '87; Young and Badyaev, 2007).

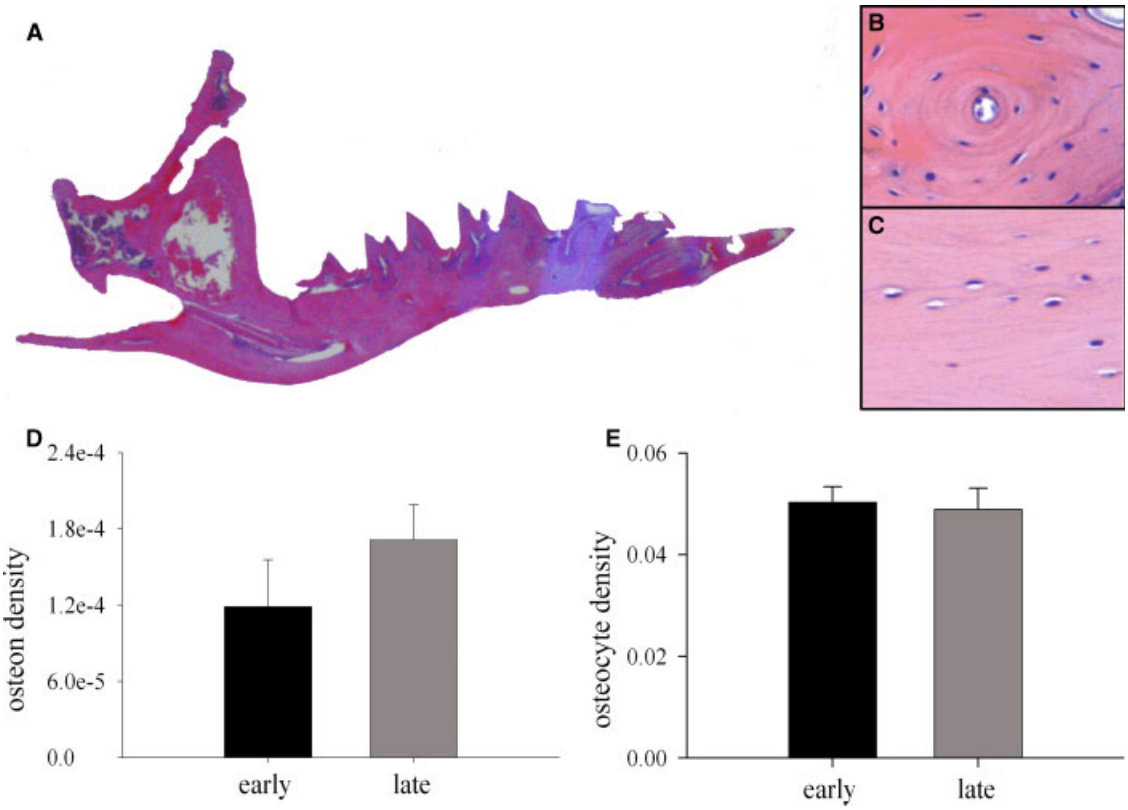

Figure 5. Bone remodeling in the early- and late-ossifying regions of the *Sorex monticolus* mandible. (**A**) Sagittal section of the mandible stained with hematoxylin (blue) and eosin (red). Stained sections were used to identify and measure density of secondary osteons (**B**) and osteocytes (**C**). Early-and late-ossifying regions of the mandible show equivalent levels of bone remodeling; density of (**D**) secondary osteons $U = 141$, $P = 0.15$, and (**E**) osteocytes, $U = 202$, $P = 0.98$ did not differ between the two regions. Shown are means $\pm$ 1 s.e.

Development, Function, and Evolutionary Diversification in the Mandible

To examine whether the muscle–bone developmental interactions in the late-ossifying mandibular region influence diversification across shrew species (Badyaev and Foresman, 2000; Young et al., 2007), we examined correspondence between directions of species divergence in mandibular morphology and intraspecific patterns of muscle-induced mandibular variation across nine closely related species of shrews—*S. cinereus, S. fumeus, S. haydeni, S. hoyi, S. monticolus, S. pacificus, S. palustris, S. trowbridgii, and S. vagrans*. We documented that first, interspecific variation in size was greater in the late- vs. early-ossifying region of the mandible (Fig. 6A and B). High interspecific variation in size of the late-maturing region of the mandible might reflect differences among taxa in diet, because size variation in the late-ossifying mandibular region was closely associated with both bite force (Fig. 3C; see also Young et al., 2007) and muscle activity (Table 5) within species. Second, the patterns of interspecific variation in shape of the late-maturing mandibular regions were highly concordant with the within-species directions of muscle-induced morphological variation (Fig. 6C and D). Within species, variation in the late-maturing mandibular region resulted in individual variation in bite force (Fig. 3D), suggesting that the major axis of species divergence occurs along the lines delineated by intraspecific effects of foraging. Thus, divergence among these taxa in diet and associated muscle activity might direct the development of adaptive morphological diversification, and corroborates previous observations of the impact of environmental variation on diversification of foraging morphology of *Sorex* shrews (Badyaev and Foresman, 2000; Young and Badyaev, 2006).

Taken together, these results suggest that epigenetic regulation of bone formation can provide a common developmental pathway for both local adaptation and diversification in mandible morphology. We found that morphological diversification across species was concordant with intraspecific patterns of adaptive variation likely induced through muscle–bone interactions during development. Second, our results suggest that the late-ossifying region of the mandible was more influenced by functionally important muscle activity and that the resulting

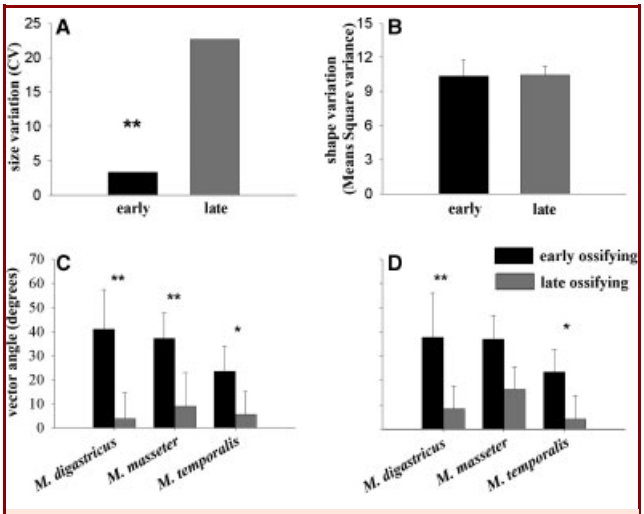

Figure 6. Patterns of interspecific divergence in the early- and late-ossifying mandibular regions. **(A)** Across species, mandible size variation (interspecific coefficients of variation, CV) was higher in the late-ossifying regions compared with the early-ossifying regions (Levene's test, $F = 10.5$, $P < 0.01$). **(B)** Interspecific shape variation (Means Square variance of landmark variation among species) did not differ between the early- and late-ossifying regions ($t = 0.17$, $P = 0.86$). **(C–D)** The directionality of shape diversification across species was more concordant with intraspecific variation in mandible shape due to muscle loading in the late-ossifying mandible than the early-ossifying mandible before (C) and after (D) correcting for allometric variation in shape. Concordance is measured as the angle between the first canonical variate of mandible shape variation across taxa and the single PLS axis of intraspecific mandible shape variation associated with loading of each muscle (mean $\pm$ bootstrapped s.d.). More acute angles indicate higher similarity. (C) Before correcting for allometric variation in shape, intraspecific variation associated with loading of all three muscles (*M. digastricus*: $t = 4.4$, $P < 0.01$; *M. masseter*: $t = 3.4$, $P < 0.01$; *M. temporalis:* $t = 2.9$, $P < 0.05$) was highly concordant with interspecific shape variation of the late ossifying ($CV1\lambda = 0.02$, $P < 0.01$) but not early ossifying ($CV1\lambda = 0.06$, $P < 0.01$) region of the mandible. (D) After correcting for allometric variation in shape, intraspecific variation associated with loading of the *M. digastricus* ($t = 3.2$, $P < 0.01$) and *M. temporalis* ($t = 3.5$, $P < 0.01$) were highly concordant with interspecific shape variation of the late-ossifying region (late $CV1\lambda = 0.11$, $P < 0.01$) but not early ossifying region ($CV1\lambda = 0.17$, $P < 0.01$) of the mandible. Although the effects of *M. digastricus* tended to be more concordant with interspecific shape variation in the late-ossifying region, this relationship was not significant ($t = 2.22$, $P = 0.06$). One asterisk indicates $P < 0.05$ and two asterisks indicate $P < 0.01$.

form of the late-ossifying region of the mandible closely covaried with bite force. Further, plasticity of the late-ossifying region of the mandible may be an important source of variation enabling morphological divergence across shrew species experiencing distinct ecological requirements of foraging. This role of muscle-induced plasticity combined with the well-established, conserved role of muscle–bone interaction in skeletal development suggests that changes in plasticity of skeletal traits may evolve through shifts in the timing of bone development, or other mechanisms (e.g., prolonged bone remodeling) that extend the duration of skeletal sensitivity to ecologically relevant functional demands.

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