

Morphological diversity and ecological similarity: versatility of muscular and skeletal morphologies enables ecological convergence in shrews

Rebecca L. Young^{*,†}, Michael J. Sweeney and Alexander V. Badyaev

Department of Ecology and Evolutionary Biology, University of Arizona, 1041 E. Lowell, Tucson, AZ 85721, USA

Summary

1. Ecological convergence in morphology among taxa of distinct evolutionary histories is a common illustration of the efficacy of natural selection. Ecological convergence is often enabled by functional redundancy of complex morphological structures, such that modification of existing morphologies in response to similar functional requirements can lead to the development and evolution of morphological diversity. Thus, studies of the mechanisms that enable the development of similar adaptations in taxa with distinct morphologies provide important insights into both the evolution of past adaptations and patterns of future evolutionary divergence.

2. Here, we examine mechanisms that have enabled ecological convergence in foraging morphology among four geographically isolated and morphologically distinct populations of shrews: south-eastern Arizona and north-central New Mexico populations of the montane shrews (*Sorex monticolus*) and northern California and north-central Montana populations of the vagrant shrew (*S. vagrans*).

3. We show that despite overlap in diet, populations had distinct skeletal and muscular morphologies of the mandible. This association between ecological convergence and morphological uniqueness among populations was enabled by versatility of foraging morphologies that generated similar functional outputs.

4. In addition, we found that populations exhibited unique skeletal and muscular correlations with diet suggesting that distinct muscular and morphological components of the complex foraging apparatus can be used for a particular resource. This result corroborates a previous finding that extensive modularity in mandibular development allows diverse morphologies to generate equivalent functions and utilize similar resources across taxa.

5. *Synthesis.* We conclude that the observed functional and ecological convergences resulted from population-specific musculoskeletal interactions, and suggest that the differences in skeletal and muscular morphologies observed among these populations reflect evolved differences in plasticity of the skeletal and muscular components of the mandible.

Key-words: ecological convergence, mandible, morphological versatility, plasticity, *Sorex*

Introduction

The distinct evolutionary histories and developmental properties of taxa affect responses of morphological traits to convergent selection. Because of the versatility of developmental variation in complex phenotypes, convergent selection

can result in distinct adaptive morphologies (Gould 1989; Losos *et al.* 1998; Langerhans & DeWitt 2004). Specifically, the complexity of morphological traits can allow changes in different components of a composite structure to produce similar functions (e.g. see Alfaro, Bolnick & Wainwright 2005; Young, Haselkorn & Badyaev 2007) enabling versatility in response to selection (Vermeij 1973). Although empirical evidence for the commonality of morphological versatility enabling diversification among taxa utilizing similar resources continues to grow (Wainwright *et al.* 2005), it remains unclear how morphological adaptations get

[†]Present address: Department of Ecology and Evolutionary Biology, Yale University, 165 Prospect St., New Haven, CT 06520, USA.

*Correspondence author. E-mail: rebecca.youngbrim@yale.edu

partitioned to distinct components of a composite trait in different populations and taxa.

Variation in skeletal traits is primarily generated through changes in the rate and timing of growth and differentiation (Atchley 1993; Atchley & Hall 1991; Smith & Hall 1990; Skerry 2000) under the influence of internal and external stresses (Frost 1987; Herring 1993; Huiskes 2000; Skerry 2000; Moore 2003). Modification in use of a skeletal trait often requires adjustments of the musculature (e.g. resulting in increased strength output or muscle firing speed; Liem 1978; Flück 2006). These modifications in the musculature or in muscle activity can subsequently induce morphological variation through skeletal plasticity – as muscle stimulation influences bone growth, development and remodelling (Enlow 1963; Lanyon 1984; Frost 1987; Lanyon 1993). Populations may differ in sensitivity to muscle stimulation as the evolution of phenotypic plasticity depends on both the within-generation reliability and across-generation predictability of the environment (Levins 1968; Moran 1992; Padilla & Adolph 1996; Young & Badyaev 2007). Thus, across populations and species, selection on trait function over time can result in evolved differences in reaction norms (Scheiner 1993; Schlichting & Pigliucci 1998; West-Eberhard 2003; Badyaev 2005) and subsequently the development of distinct morphological adaptations. Indeed, the intensity, duration and frequency of muscle stimulation required to induce a plastic response in skeletal traits (e.g. a change in growth or induction of remodelling) often differ across populations (e.g. see Duncan & Turner 1995; Heaney 1995; Parfitt 1997).

In this study, we characterized musculoskeletal morphology and function (i.e. bite force) of the foraging apparatus in four populations and two species of shrews (*Sorex monticolus* Merriam, 1890: 43 and *Sorex vagrans* Baird, 1857:15) that have similar diets. In shrews, similarities among taxa in ecology and function are not indicative of similarities in mandible morphology as distinct mandible morphologies can generate equivalent functions (Young, Haselkorn & Badyaev 2007). In addition, development of the shrew mandible is highly sensitive to environmental stressors such as interspecific competition (Foresman & Badyaev 2005) and environmental disturbances (Zakharov *et al.* 1991; Pankakoski, Koivisto & Hyvrinen 1992; Badyaev, Foresman & Fernandes 2000). Specifically, plastic response of the mandible to muscle stimulation is an important mechanism of local adaptation in this system (Badyaev, Foresman & Young 2005; R.L. Young & A.V. Badyaev, unpublished). Thus, differences in morphological adaptation to a common diet may be driven by evolved differences among populations in skeletal response to muscle stress.

Here, we show that despite similarity in diet and overlap in the estimated bite force, species and populations differ in mandible muscular and skeletal morphology. We examined correlations among the mandible and the major jaw muscles (*Musculus temporalis* Edgeworth 1935, *Musculus masseter* Gaughran 1954 and *Musculus digastricus* Sharma 1958) and show that functional equivalence of distinct

morphologies among populations is enabled by population-specific patterns of muscular and musculoskeletal interactions. Finally, we suggest that the population-specific morphology likely reflects evolved differences in plasticity that generates distinct skeletal responses to muscle stress among species and populations.

Materials and methods

DATA COLLECTION

Diet assessment

In accordance with the standards of animal care and use (IACUC No. 04-090), 40 shrews (*S. monticolus* and *S. vagrans*) were captured in four geographically isolated populations in south-eastern Arizona, north-eastern California, north-central Montana and north-central New Mexico. Upon capture, individuals were sacrificed and fixed in 10% buffered formalin overnight and stored in 70% ethanol. After fixation, we removed contents from both the stomach and intestine and separated the insect cuticle pieces (hard contents) from the remaining contents (soft contents). Using these materials, three characteristics of individual diet were assessed. First, we documented the relative amount of hard insect parts consumed by measuring the proportion of dry mass of insect cuticle to the remaining contents of the stomach and intestine. Second, we characterized hardness of an individual's diet by measuring mean thickness of the cuticle present in the stomach and intestines. Thickness of the cuticle is an indicator of strength, stiffness and toughness of the insect (Evans & Sanson 2005), and thus might represent how difficult an insect is to process as a prey item. A sample of 10 cuticle pieces from each individual was used for the thickness measurements. Several individuals ($n = 11$) had <10 total cuticle pieces, for these individuals all cuticle pieces were measured. Cuticle thickness was measured using a digital point micrometre (Fowler Inc., Newton, MA, USA) by placing the cuticle on the point of the micrometre and measuring thickness of the flattest plane of the cuticle piece (after Evans & Sanson 2005). Variation in diet hardness was the variance of the sample of 10 cuticle thickness measurements. In addition to these individual measures, overall diet was defined as the first principal component of the proportion of cuticle, mean cuticle thickness and cuticle thickness variance. Stomach and intestine data were available for only four *S. vagrans* shrews from north-central Montana. Thus, the Montana population was not included in analyses involving diet; however, data from these individuals suggest that the Montana population shares these characteristics of diet (proportion of insect cuticle = 0.12; mean cuticle thickness = 0.002; variance in cuticle thickness = 1.9E-5, see below) with the Arizona, California and New Mexico populations.

Mandible muscular and skeletal morphology

We measured mass, fibre length and attachment location for the *M. digastricus*, *M. masseter* and *M. temporalis*, three muscles with known importance for prey capture and processing (Fig. 1; Dötsch 1985, 1994; R.L. Young & A.V. Badyaev, unpublished). Muscles were dissected off of both the left and right mandibles, weighed (with 0.01 mg resolution) using a Mettler Toledo AB135-S/FACT balance (Columbus, OH, USA), immersed in 40% nitric acid (HNO₃) until muscle fibres separated (24–30 h) and stored in a 50% aqueous glycerol solution. Muscle fibres were photographed under 10–12.5× magnifications

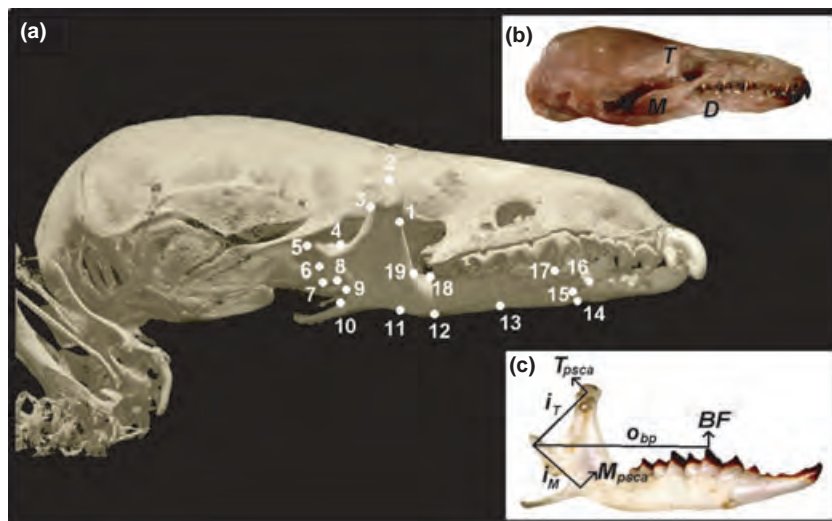


Fig. 1. Skeletal (a), muscular (b) features of the shrew-foraging apparatus. (a) Nineteen landmarks were used to characterize mandible form (i.e. size and shape). (b) Three muscles critically important for prey capture and prey processing, *Musculus temporalis* (T), *Musculus masseter* (M) and *Musculus digastricus* (D) were used to describe the musculature of the foraging apparatus. Letters identifying each muscle overlay the general area of muscle attachment on the mandible. (c) Components of the lever model include two in-force arms (i_T and i_M), their associated muscular forces (T_{psca} and M_{psca}), and an out-force arm (o_{bp}). Arrows indicate the direction of force input (muscle forces) or output (bite force). See Materials and methods for additional description of bite force estimation.

using a Leica DC 300 (Bannockburn, IL, USA), sizes were standardized using a ruler photographed along with the fibres. Fibre length of each muscle was estimated as the mean length of 20 unique fibres. We calculated the physiological cross-section area (cm^2) of each muscle as the quotient of muscle mass (g) and mean fibre length (cm) (after Herrel, Aerts & De Vree 1998). Physiological cross-section areas of the right and left mandibles were averaged. The average physiological cross-section areas were used for all analyses and for our estimation of bite force (see below). Because physiological cross-section area responds to changes in physical activity (Narici *et al.* 1989) and is a metric of the maximum muscle tension (Close 1972), it can be used to examine changes in the muscle that may be important for biting or prey processing, and it provides a measure of the stimulation experienced by the attached skeletal trait.

Shrew mandibles were cleaned and photographed under 10 \times magnifications using a Leica DC 300 (Bannockburn, IL, USA), image sizes were standardized using a ruler photographed along with the mandibles. From mandible images, we obtained x and y coordinates of 19 homologous morphological landmarks distributed across the mandible (Fig 1; Badyaev & Foresman 2000; R.L. Young & A.V. Badyaev, unpublished). Each mandible image was measured twice and the landmarks were averaged to improve measurement errors. All data were collected using tpsDig2 (Rohlf 2005). To separate variation due to mandible size and shape, all specimens were scaled to unit centroid size and landmark configurations were aligned from all landmarks, species, and individuals using a single Procrustes superimposition (generalized orthogonal least-squares fit, Rohlf & Slice 1990; Badyaev & Foresman 2000). Finally, a principal component analysis of landmark configurations (Procrustes coordinates) was used to summarize the major axes of mandible shape variation.

Estimation of bite force

To assess mandible function, we estimated potential bite force for all individuals using a lever model (after Thomason 1991). Bite force (BF) was estimated as:

$$BF = 2 \times (T_{psca} \times i_T + M_{psca} \times i_M) / o_{bp},$$

where T_{psca} and M_{psca} are the physiological cross-section areas of the *M. temporalis* and *M. masseter* respectively (Fig. 1b,c),

multiplied by a force conversion factor ($C = 25N$, after Herzog 1994). i_T and i_M are the in-force arm lengths, measured as the distance from the mandible joint to the centre of the *M. temporalis* and *M. masseter* mandible attachment sites (Fig. 1c). The in-force (numerator) was multiplied by two to account for simultaneous firing of the left and right musculature. Because the in-force arms were measured to the centre of the muscle attachment sites, the vectors of muscle in-force were assumed to act perpendicularly (Fig. 1c, arrows). o_{bp} is the out-force arm length, measured as the distance from the mandible joint to the bite point (Molar 1, Fig. 1c). This metric of function provides an estimate of bite force when both muscles are firing at their maximum tension and thus provides an estimate of maximum bite force.

DATA ANALYSIS

Variation in diet, morphology, musculature and function among populations

To examine the within and among species concordance of diet, mandible morphology and musculature, we first used canonical discriminant analysis to summarize variation in diet, morphology and musculature within each population. Mahalanobis distances were calculated between all populations for each variable. To illustrate differences in mandible morphology we plotted the distribution of mandible sizes (centroid size) and shapes (PC1 and PC2) for each species. We tested for both intra- and interspecific divergence in the three diet characteristics (i.e. proportion of cuticle, mean cuticle thickness and cuticle thickness variance), mandible morphology, musculature and estimated bite force using ANOVA, and significance for individual population comparisons was assessed using Tukey's test for multiple comparisons. Diet, muscular and bite force data were arcsine or log-transformed to achieve normal distribution.

Relationships among diet, function and musculoskeletal morphology

To examine the relationships between mandible function, musculoskeletal morphology and diet, we first assessed the correlations between diet and mandible morphology as well as diet and mandible musculature using multiple regression, where diet was the

independent variable and mandible form (i.e. centroid size, PC1 of mandible shape and PC2 of mandible shape) or physiological cross-section area of each muscle were the dependent variables. To assess the relationship between diet and the combined musculature, we performed a regression of the PC1 of the physiological cross-section areas of all three muscles on diet. Next, we examined the correlation between diet and estimated bite force using multiple regression, where each characteristic of diet (i.e. proportion of insect cuticle, cuticle thickness and variation in cuticle thickness) was the dependent variable and estimated bite force was the independent variable. In addition, we regressed the overall diet (i.e. PC1 of diet) on estimated bite force. Finally, to assess differences among populations in muscular and musculoskeletal interactions, we first described muscle interactions in each population by comparing the physiological cross-section areas of all three muscles using regression analysis. Second, we characterized musculoskeletal interactions in each population using multiple regression, where morphological traits (centroid size, PC1 or PC2 of shape) were the dependent variables and physiological cross-section areas of all muscles were the independent variables.

Results

INTER- AND INTRASPECIFIC CHARACTERISTICS OF DIET, MUSCULOSKELETAL MORPHOLOGY AND FUNCTION

Both species and populations overlapped in the aspects of diet measured in this study (Fig. 2a). There were no significant differences in the relative amount of hard-bodied insects con-

sumed – measured as the proportion of insect cuticle in the stomach and intestine, the mean thickness of insect cuticle, or the variance in insect cuticle thickness among populations (Fig. 3a–c) or species – proportion of cuticle in the diet: $F = 1.8$, $P = 0.2$; mean thickness of cuticle: $F = 0.4$, $P = 0.5$; variance in cuticle thickness: $F = 0.2$, $P = 0.7$. Moreover, overall diet – PC1 of the three characteristics of diet describing 45.8% of diet variation – did not differ among populations (Table 1; Fig. 2a).

We found significant overlap in estimated bite force both within and between species (Fig. 4a). Mean estimated bite force differed among all the populations ($F = 7.56$, $P < 0.01$; Fig. 4b); however, only the California population of *S. vagrans* and the Arizona population of *S. monticolus* differed significantly in estimated bite force (Fig. 4b).

Although all populations and both species consumed similar proportions of hard-bodied insects, had similar hardness of diet and most populations overlapped in bite force, they differed significantly in mandible muscular and skeletal morphology (Fig. 2b,c). We found significant interspecific, but not intraspecific, differences in the musculature (Table 1; Figs 2b and 3d–f). The Arizona and California populations of *S. monticolus* and *S. vagrans* respectively differed in physiological cross-section areas of the *M. digastricus* (Fig. 3d), and physiological cross-section area of *M. temporalis* differed in all interspecific population comparisons (Fig. 3f). Overall mandible musculature, measured as PC1 of the physiological

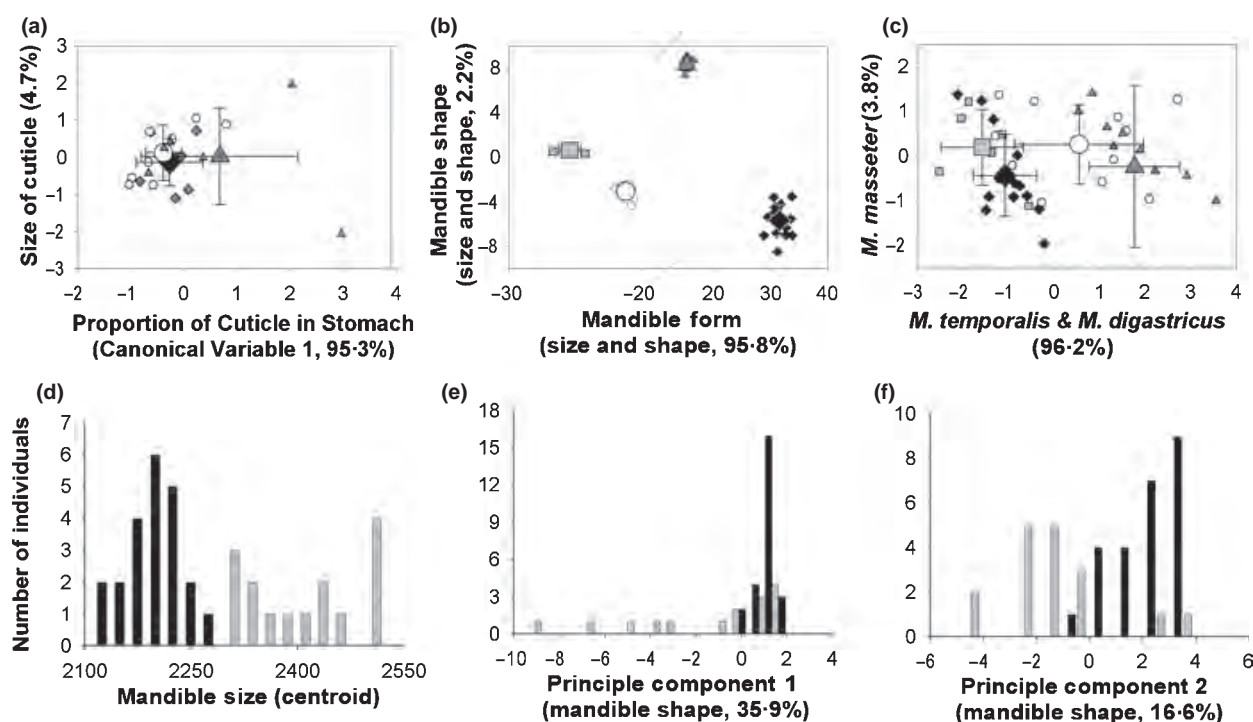


Fig. 2. Canonical discriminant analysis of diet (a), mandible morphology (b) and musculature (c) among populations. Abscissa is the first canonical axis; ordinate is the second canonical axis. Shown are the means \pm 1 standard deviation for each population for the first two canonical axes. Black diamonds and dark grey triangles show the Arizona and New Mexico populations of *Sorex monticolus* respectively. White circles and light-grey squares show the California and Montana populations of *Sorex vagrans*. (d–f) Characteristics of mandible form across four geographically isolated populations of two species of soricid shrews (*S. monticolus* and *S. vagrans* in black and grey bars, respectively).

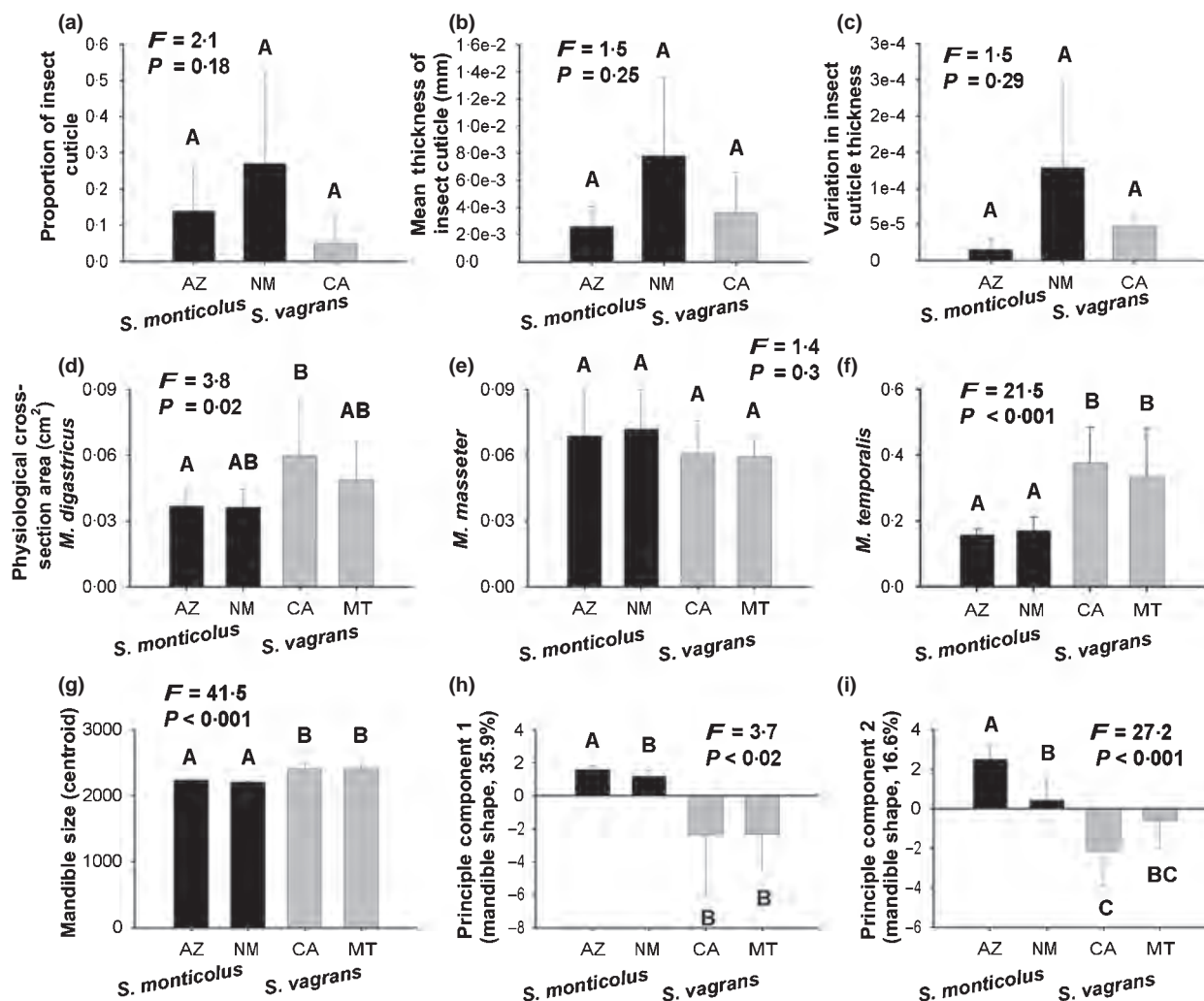


Fig. 3. Comparisons of diet (a–c), musculature (d–f) and mandible form (g–i) across populations. Plotted are population means for each variable, error bars indicate ± 1 standard deviation. Differences among populations in each variable (at $\alpha \leq 0.05$) are indicated by differences in the associated letter.

Table 1. Morphological differences despite use of similar resources in two populations of *Sorex monticolus* – south-eastern Arizona (AZ) and north-central New Mexico (NM) – and two populations of *S. vagrans* – northern California (CA) and north-central Montana (MT)

Variable	Intraspecific comparisons		Interspecific comparisons			
	AZ-NM	CA-MT	AZ-CA	AZ-MT	CA-NM	NM-MT
Form	4.58**	2.15*	44.9**	29.4**	30.5**	20.3**
Musculature	0.92	0.58	7.62**	4.37**	9.45**	5.43**
Diet	0.91	.	0.07	.	1.1	.

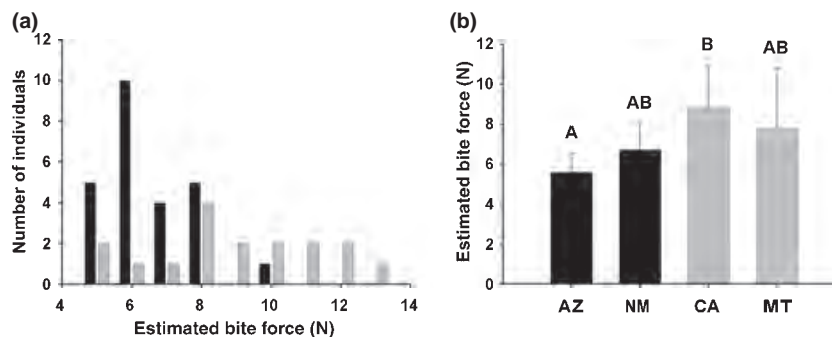
Shown are the Mahalanobis distances between populations in the first canonical axes of mandible form (size and shape combined), musculature and diet. Populations exhibiting significant differences are highlighted in bold.

*Significant at $\alpha = 0.05$.

**Significance at $\alpha = 0.01$.

cross-section areas of all three mandible muscles, was similar between populations of the same species, but differed between species (Table 1; Fig. 2c). Skeletal morphology, described as mandible size (centroid size) and mandible shape (the first two principal components of the Procrustes shape coordinates), differed between species, but not among populations (Table 1; Fig. 2b). This intraspecific similarity in mandible form was a result of similarities in mandible size among populations of the same species. In *S. monticolus*, populations did not differ in mandible size (Fig. 3g); however, they differed in mandible shape as described by the first two principal components (52.5% of mandible shape variation; Fig. 3h,i). Similarly, in *S. vagrans*, populations did not differ in mandible size (Fig. 3g), but differed in the aspects of mandible shape described by PC2 (16.6% of mandible shape variation; Fig. 3i). The patterns of mandible shape variation due to PC1 and PC2 were complex; however, in general, mandible shape variation due to PC1 was associated with a shortening of the

Fig. 4. Comparison of mandible function across species (a) and populations (b). (a) Shown is the distribution of bite forces in *Sorex monticolus* (black bars) and *Sorex vagrans* (grey bars). (b) Plotted are the population means for estimated bite force, error bars indicate ± 1 standard deviation. Differences among populations (at $\alpha \leq 0.05$) are indicated by differences in the associated letter.



mandible body as well as an increase in the angle between the coronoid process and the mandible body. Shape variation due to PC2 was associated with a shortening of the ramus and the coronoid process.

POPULATION-SPECIFIC RELATIONSHIPS BETWEEN DIET, MUSCULOSKELETAL MORPHOLOGY AND FUNCTION

The relationship between diet and morphology differed among all populations (Tables 2 and 3). We found that PC1 of the physiological cross-section areas of all muscles (56.3% of the variation) was correlated with diet in New Mexico's population of *S. monticolus* and physiological cross-section area of *M. masseter* correlated with diet in California's population of *S. vagrans* (Table 2); however, in the Arizona population of *S. monticolus*, there were no significant correlations between musculature and diet. For mandible form, only one population exhibited a significant correlation with diet. In New Mexico's population of *S. monticolus*, we found that PC1 of mandible shape (35.9% of the variation) and diet were correlated suggesting a diet-specific adaptation of skeletal morphology in this population.

The relationship between estimated bite force and diet differed among all populations (Table 4). Estimated bite force correlated with PC1 of diet (45.8% of the variation in diet) in the New Mexico population and with the proportion of insect

Table 2. The correlations between diet and mandible musculature across populations of *Sorex monticolus* and *Sorex vagrans*

Physiological cross-section area (cm ²)	<i>Sorex monticolus</i>		<i>Sorex vagrans</i>
	AZ	NM	CA
<i>Musculus digastricus</i>	-0.01	-0.77	-0.33
<i>Musculus masseter</i>	0.34	-0.41	0.84*
<i>Musculus temporalis</i>	0.31	-0.67	-0.17
PC1 of muscle	0.36	-0.91*	-0.35

Shown are the partial regression coefficients of the multiple regression of the physiological cross-section area of each muscle (*Musculus digastricus*, *Musculus masseter* and *Musculus temporalis*) on diet. The bolded value indicates a non-significant trend at $\alpha = 0.06$. Regression of the first principal component of the physiological cross-section areas of all three muscles on diet was performed independently.

*Significance at $\alpha = 0.05$.

cuticle found in the stomach and intestine in the California population. There were no significant correlations between diet and mandible function in the Arizona population.

Population-specific musculoskeletal interactions

To examine population differences in muscle stress and the correlation between muscle stress and mandible morphology, we compared the pairwise relationships between the physiological cross-section areas of all three muscles (Fig. 5) and the relationship between the physiological cross-section areas of

Table 3. The relationship between diet and mandible morphology across populations

Morphology	<i>Sorex monticolus</i>		<i>Sorex vagrans</i>
	AZ	NM	CA
Centroid size	0.01	0.11	0.21
Shape PC1	-0.1	-0.76*	0.19
Shape PC2	-0.18	-0.12	-0.19

Shown are the partial regression coefficients of a multiple regression of mandible form (i.e. size and shape) on diet. Populations exhibiting significant differences are highlighted in bold.

*Significance at $\alpha = 0.05$.

Table 4. The relationship between mandible function and diet across populations

Diet	<i>Sorex monticolus</i>		<i>Sorex vagrans</i>
	AZ	NM	CA
Proportion of insect cuticle	-0.20	-0.62	-0.79*
Thickness of insect cuticle	0.29	0.1	0.11
Variation in cuticle thickness	0.22	0.62	0.12
PC1 of diet	-0.05	-0.9*	0.03

Shown are the partial regression coefficients of a multiple regression of each characteristic of diet assessed (proportion of insect cuticle in the stomach and intestine contents, thickness of insect cuticle and variation in insect cuticle thickness) on estimated bite force. Regression of the first principal component of the three diet characteristics on estimated bite force was performed independently. Populations exhibiting significant differences are highlighted in bold.

*Significance at $\alpha = 0.05$.

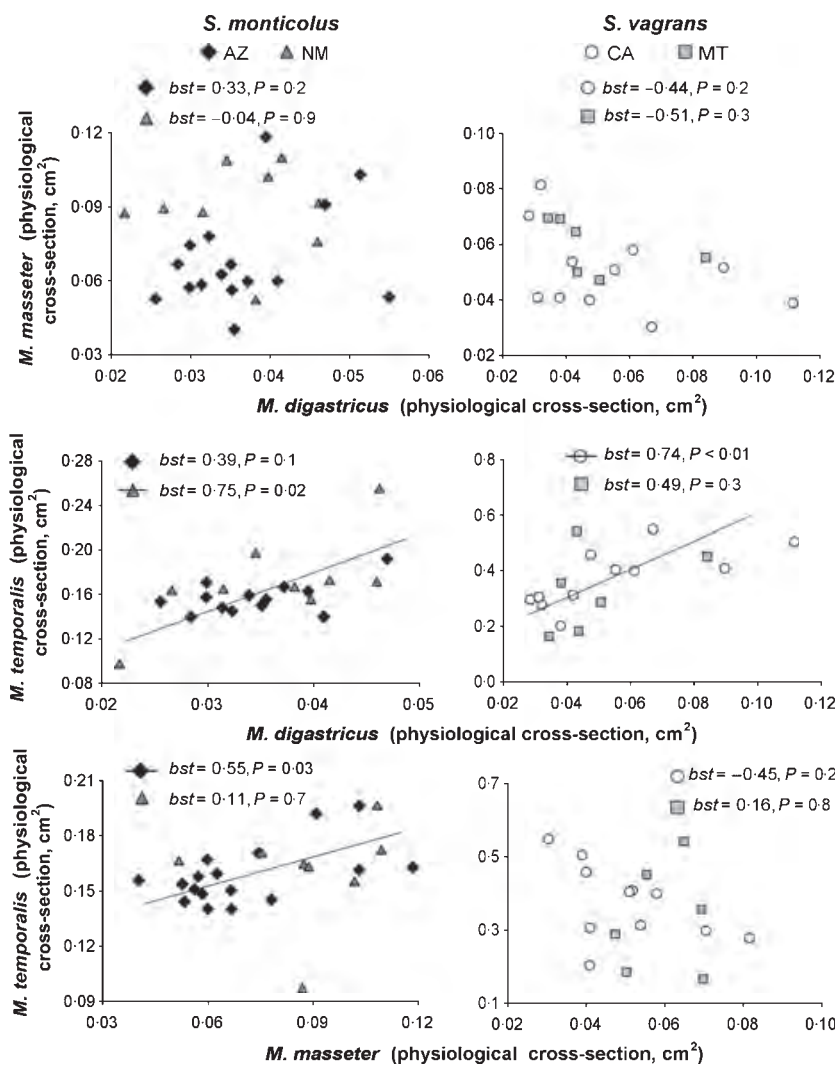


Fig. 5. Population-specific muscular interactions for *Sorex monticolus* (left column) and *Sorex vagrans* (right column). Black diamonds and dark grey triangles show the Arizona and New Mexico populations respectively. White circles and light grey squares show the California and Montana populations. Regression lines indicate significant correlations (at $\alpha \leq 0.05$).

all three muscles and mandible size and shape (Table 5). Populations differed in muscle–muscle correlations suggesting that across populations jaw function favours the use of different muscle combinations. In *S. monticolus*, we found that an increase in physiological cross-section area of the *M. masseter* was correlated with an increase in the physiological cross-section area of *M. temporalis* in Arizona's population (Fig. 5), and in New Mexico's population, we found that a larger *M. digastricus* was correlated with a larger *M. temporalis* (Fig. 5). In *S. vagrans*, the physiological cross-section areas of the *M. digastricus* and *M. temporalis* were correlated in the California population (Fig. 5), but not the Montana population.

Populations differed in musculoskeletal interactions; in Arizona's population of *S. monticolus*, both mandible size and shape correlated with morphology of several muscles (Table 5). Mandible size was correlated with PC1 of all three muscle physiological cross-section areas and variation in shape was correlated with the physiological cross-section areas of the *M. temporalis*, *M. digastricus* and PC1 of all three muscles (Table 5). In the New Mexico population of *S. monticolus*, small adjustments of mandible shape (described by

PC2 of mandible shape variation) were correlated with PC1 of the physiological cross-section areas of all three muscles (Table 5). In the California population of *S. vagrans*, mandible shape variation, but not mandible size, was correlated with the physiological cross-section area of the *M. digastricus* and *M. temporalis* muscles (Table 5). In Montana's population of *S. vagrans*, mandible muscular and skeletal morphologies did not correlate (Table 5).

Discussion

Convergent use of resources commonly results in development of morphological similarities among taxa (Simpson 1953; Mayr 1963). However, for composite traits, flexibility in development combined with functional redundancy of distinct morphologies allows for diverse responses to similar selection that can produce morphological diversity among taxa exploiting similar resources. In this study, geographically isolated shrew populations varied in both mandible form and musculature (Figs 2 and 3; Table 1). Despite their morphological differences, shrews in these populations consumed similar diets of insects with hard exoskeletons (Fig. 2a and

Table 5. Differences among populations in musculoskeletal interactions

Morphology	Physiological cross-section area (cm ²)	<i>Sorex monticolus</i>		<i>Sorex vagrans</i>	
		AZ	NM	CA	MT
Centroid size	<i>M. digastricus</i>	0.41	-0.49	0.24	0.27
	<i>M. masseter</i>	0.29	-0.11	-0.12	0.82
	<i>M. temporalis</i>	0.04	0.06	-0.47	0.05
	PC1 of all muscles	0.55	-0.49	-0.16	0.23
Shape PC1	<i>M. digastricus</i>	0.28	-0.07	-1.01**	-0.4
	<i>M. masseter</i>	0.21	-0.1	0.19	-0.85
	<i>M. temporalis</i>	-0.63	0.68	1.17*	0.04
	PC1 of all muscles	0.05	0.32	0.21	-0.25
Shape PC2	<i>M. digastricus</i>	-0.46*	0.1	1.14*	0.12
	<i>M. masseter</i>	-0.21	-0.47	0.06	0.68
	<i>M. temporalis</i>	-0.24	-0.4	-1.07*	-0.3
	PC1 of all muscles	-0.58*	-0.62	0.04	-0.2

Shown are the partial regression coefficients of a multiple regression of physiological cross-section area of all muscles (*Musculus digastricus*, *Musculus masseter* and *Musculus temporalis*) on mandible size (centroid size) and shape (PC1 and PC2). The bolded values indicate a non-significant trend at $\alpha = 0.06$. Regression of the first principal component of muscle physiological cross-section area on diet was performed independently.

*Significance at $\alpha \leq 0.05$.

**Significance at $\alpha \leq 0.01$.

Table 1) and had similar estimated bite forces (Fig. 4). The ability of distinct morphologies to generate similar functions and utilize similar resources requires that components of the complex shrew-foraging apparatus vary independently. In fact, morphological diversification across populations and taxa utilizing similar resources is thought to be a consequence of modularity – or semi-independence of components of a complex trait (Vermeij 1973; Alfaro, Bolnick & Wainwright 2004) – allowing different features of a trait (e.g. distinct skeletal or muscular elements) to adapt to similar conditions across groups (Alfaro, Bolnick & Wainwright 2005; Young, Haselkorn & Badyaev 2007). Our results indicate that each population exhibited a unique combination of skeletal and muscular characteristics (Figs 2 and 3). These distinct musculoskeletal morphologies may reflect population-specific solutions to common functional requirements of the jaw. For example, despite the size differences between the species (e.g. centroid size; Fig. 3g), species had similar physiological cross-section areas of the *M. masseters* (Fig. 3e) – a primary muscle generating both biting force (Fig. 1c; R.L. Young & A.V. Badyaev, unpublished) and jaw action associated with prey grinding in shrews (Dötsch 1994). The increase in the physiological cross-section area of the *M. masseter* in the smaller *S. monticolus* jaw allows these distinct mandible morphologies to generate bite forces similar to the larger *S. vagrans* (Fig. 4) and thus utilize equivalent food resources (Figs 2 and 3). Moreover, we found that populations differed in their muscular and skeletal correlations with diet (Tables 2 and 3) suggesting that there are multiple distinct components of the shrew-foraging apparatus that can adapt for use of a

particular resource. For example, both the California and New Mexico populations exhibited correlations between musculature and diet (Table 2); however, the role of each muscle in this correlation differed between the populations (Table 2). Overall, these results corroborate findings that modularity of the shrew-foraging apparatus enables versatility of distinct morphologies to generate equivalent functions (Young, Haselkorn & Badyaev 2007).

Although the population-specific morphologies characterized in this study are expected to generate equivalent functions (based on estimated bite forces), one population – the Arizona population of *S. monticolus* – exhibited a reduced bite force (Fig. 4b) despite its overlap in diet with the other populations (Figs 2a and 3a–c). Interestingly, unlike the other populations, in Arizona, there were no significant correlations between bite force and the aspects of diet assessed in this study (Table 4) or between diet and musculoskeletal characteristics of the mandible (Tables 2 and 3). Despite a lack of correlations found between diet and musculoskeletal morphology, the Arizona population exhibited several muscular and musculoskeletal interactions (Fig. 4; Table 5). These interactions suggest that the mandible may be adapted for some other functional requirement or that muscle stress associated with other mandible functions may influence mandible morphology in this population (e.g. jaw opening, defence or echolocation, Gould, Negus & Novick 1964).

Consistent with previous findings, our results indicate that similar functional requirements can be achieved by distinct morphologies; however, the developmental mechanisms that result in channelling of developmental variation to different components of a complex trait even among closely related populations remain unclear. Because of the high sensitivity of shrew mandible morphology to environmental inputs (Zakharov *et al.* 1991; Pankakoski, Koivisto & Hyvrinen 1992; Badyaev & Foresman 2000), especially those mediated by muscle–bone interactions (Badyaev, Foresman & Young 2005; R.L. Young & A.V. Badyaev, unpublished), we predicted that the morphological differences among populations could result from systematic difference in skeletal plasticity in response to stress of the mandible musculature. We found that populations differed in both muscle–muscle (Fig. 5) and musculoskeletal interactions (Table 5). Such differences may reflect distinct patterns of phenotypic plasticity among populations. Interestingly, we found no evidence of musculoskeletal interactions in the Montana population (Table 5). Because the effects of muscle stress on bone growth, development and remodelling are well-established (Enlow 1963; Lanyon 1984; Frost 1987), the absence of musculoskeletal interactions in this population is puzzling. This finding may reflect a delay in accommodation of the bone to changes in muscle stress, and further suggests that this population is less plastic (e.g. requiring greater intensity or duration in muscle stimulation to induce bone remodelling). Alternatively, this may reflect limitations of the methodology used to measure muscle stress (i.e. physiological cross-section area) as other features of the musculature (e.g. the pennation angle of the muscle fibres) influence both strength and speed of muscle

stress. For example, observations of foraging activities in several shrew species (*S. cinereus*, *S. monticolus* and *S. vagrans*) in Montana revealed that shrews primarily consumed the large muscular components of the insect (e.g. the flight muscles of dragonflies) leaving the remainder of the body intact (A. Badyaev, pers. observation). Capturing and processing prey in this manner may require large gape angles and rapid muscle action rather than a modulation of biting forces or grinding action of the jaw.

Differences in the type and magnitude of muscle–muscle and muscle–bone interactions associated with similar resource use and similar mandible functions across populations raise questions about the mechanisms underlying differences in plasticity. What mechanisms mediate differences in response to muscle stress across populations? How do these differences in plasticity evolve? Differences in the consistency of resource use over an individual's lifetime may influence the skeletal response to muscle stress (Robling, Castillo & Turner 2006), because the magnitude of skeletal response depends on the strength, duration, frequency and rate of muscle stimulation (Duncan & Turner 1995). Thus, populations that have diverse diets due to fluctuations in insect populations or high levels of resource competition should experience variation in muscle stress. This variation is expected to inhibit the evolution skeletal plasticity. Consistent with this expectation, we found that the population exhibiting the most variable diet – and thus potentially experiencing the most variation in muscle stress – also had the fewest significant musculoskeletal interactions (New Mexico population: Fig. 3a–c and Table 5). Beyond difference in the strength, duration or frequency of muscle stress required to induce a plastic response, ontogenetic timing can influence skeletal responses to muscle stress. Although muscle actions throughout an organism's life can influence skeletal phenotypes (especially under heavy muscle stimulation, Ryan *et al.* 1994; Firth 2006), the magnitude of skeletal response decreases with age (Parfitt 1997; Power *et al.* 2002; Ruff 2003). In shrews, variation in the timing of development across regions of the mandible determines the contribution of the mandibular region to adaptive plasticity (R.L. Young & A.V. Badyaev, unpublished) and thus population-specific skeletal plasticity may result from evolved differences in initiation, rate or duration of bone maturation (Young & Badyaev 2007).

Overall, our study shows that ecological convergence can lead to the development and evolution of phenotypic diversity even among closely related populations, and provides support for the hypothesis that diversification in response to similar natural selection is facilitated by the complexity and versatility of morphological traits and their development.

Acknowledgements

We thank K. Borgmann, R. Duckworth, K. Foresman, B. Brim and T. Young for help in the field; R. Delaney for assistance with diet analysis; A. Evans and the Badyaev Lab for discussion; and the National Science Foundation (USA), the American Association of University Women and The Packard Fellowship for funding.

References

- Alfaro, M.E., Bolnick, D.I. & Wainwright, P.C. (2004) Evolutionary dynamics of complex biomechanical systems: an example using the four-bar mechanism. *Evolution*, **58**, 495–503.
- Alfaro, M.E., Bolnick, D.I. & Wainwright, P.C. (2005) Evolutionary consequences of many-to-one mapping of jaw morphology to mechanics in labrid fishes. *The American Naturalist*, **165**, E140–E154.
- Atchley, W.R. (1993) Genetic and developmental aspects of variability in the mammalian mandible. *The Skull*, Vol. 1 (eds J. Hanken & B.K. Hall), pp. 207–247. University of Chicago Press, Chicago, IL.
- Atchley, W.R. & Hall, B.K. (1991) A model for development and evolution of complex morphological structures. *Biological Reviews*, **66**, 101–157.
- Badyaev, A.V. (2005) Stress-induced variation in evolution: from behavioral plasticity to genetic assimilation. *Proceeding of the Royal Society of London, Series B: Biological Sciences*, **272**, 877–886.
- Badyaev, A.V. & Foresman, K.R. (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, Series B: Biological Sciences*, **267**, 371–377.
- Badyaev, A.V., Foresman, K.R. & Fernandes, M.V. (2000) Stress and developmental stability: vegetation removal causes increased fluctuating asymmetry in shrews. *Ecology*, **81**, 336–345.
- Badyaev, A.V., Foresman, K.R. & Young, R.L. (2005) Evolution of morphological integration: developmental accommodation of stress-induced variation. *The American Naturalist*, **166**, 382–395.
- Baird, S.F. (1857) Exploration and surveys for a railroad route from the Mississippi River to the Pacific Ocean. War Department. Mammals. Beverly Tucker, Printer, Washington, D.C. **8**, 1–757.
- Close, R.I. (1972) Dynamic properties of mammalian skeletal muscles. *Physiological Reviews*, **52**, 129–197.
- Dötsch, C. (1985) Masticatory function in shrews (*Soricidae*). *Acta Zoologica Fennica*, **173**, 231–235.
- Dötsch, C. (1994) Function of the feeding apparatus in red-toothed and white-toothed shrews (*Soricidae*) using electromyography and cineradiography. *Advances in the Biology of Shrews* (eds J.F. Merritt, G.L. Kirkland Jr & R.K. Rose), pp. 233–239. Carnegie Museum of Natural History, Pittsburgh.
- Duncan, R.L. & Turner, C.H. (1995) Mechanotransduction and the functional response of bone to mechanical strain. *Calcified Tissue International*, **57**, 344–358.
- Edgeworth, F.H. (1935) The cranial muscles of vertebrates. Cambridge University Press, Cambridge.
- Enlow, D.H. (1963) *Principles of Bone Remodeling*. Charles C. Thomas, Springfield, IL.
- Evans, A.R. & Sanson, G.D. (2005) Biomechanical properties of insects in relation to insectivory: cuticle thickness as an indicator of insect 'hardness' and 'intractability'. *Australian Journal of Zoology*, **53**, 9–19.
- Firth, E.C. (2006) The response of bone, articular cartilage and tendon to exercise in the horse. *Journal of Anatomy*, **208**, 513–526.
- Flück, M. (2006) Functional, structural and molecular plasticity of mammalian skeletal muscle in response to exercise stimuli. *Journal of Experimental Biology*, **209**, 2239–2248.
- Foresman, K.R. & Badyaev, A.V. (2005) Developmental instability and the environment: why are some species better indicators of stress than others? *Advances in the Biology of Shrews II* (eds J.F. Merritt, S. Churchfield, R. Hutterer & B.I. Sheftel), pp. 265–272. International Society of Shrew Biologists, New York.
- Frost, H.M. (1987) Bone 'mass' and the 'mechanostat': a proposal. *The Anatomical Record*, **219**, 1–9.
- Gaughran, G.R.L. (1954) A comparative study of the osteobiology and myology of the cranial and cervical regions of the shrew, *Blarina brevicauda*, and the mole, *Scalopus aquaticus*. *Miscellaneous Papers: University of Michigan*, **80**, 1–82.
- Gould, S.J. (1989) A developmental constraint in *Cerion*, with comments on the definition and interpretation of constraint in evolution. *Evolution*, **43**, 516–539.
- Gould, E., Negus, N.C. & Novick, A. (1964) Evidence for echolocation in shrews. *Journal of Experimental Zoology*, **156**, 19–37.
- Heaney, R. (1995) Bone mass, the mechanostat, and ethnic differences. *Journal of Clinical Endocrinology and Metabolism*, **80**, 2289–2290.
- Herrel, A., Aerts, P. & De Vree, F. (1998) Ecomorphology of the lizard feeding apparatus: a modeling approach. *Netherlands Journal of Zoology*, **48**, 1–25.

- Herring, S.W. (1993) Epigenetic and functional influences on skull growth. *The Skull*, Vol. 1 (eds J. Hanken & B.K. Hall), pp. 153–206. University of Chicago Press, Chicago.
- Herzog, W. (1994) Muscle. *Biomechanics of the Musculo-Skeletal System* (eds B.M. Nigg & W. Herzog), pp. 380–390. John Wiley and Sons, Chichester, UK.
- Huiskes, R. (2000) If bone is the answer, then what is the question? *Journal of Anatomy*, **197**, 145–156.
- Langerhans, R.B. & DeWitt, T.J. (2004) Shared and unique features of evolutionary diversification. *The American Naturalist*, **164**, 335–349.
- Lanyon, L.E. (1984) Functional strain as a determinant for bone remodeling. *Calcified Tissue International*, **36**, 56–61.
- Lanyon, L.E. (1993) Osteocytes, strain detection, bone modeling and remodeling. *Calcified Tissue International*, **53**, S102–S107.
- Levins, R. (1968) *Evolution in Changing Environments*. Princeton University Press, Princeton.
- Liem, K.F. (1978) Modulatory multiplicity in the functional repertoire of the feeding mechanism in cichlid fishes. I. Piscivores. *Journal of Morphology*, **158**, 323–360.
- Losos, J.B., Jackman, T.R., Larson, A., de Queiroz, K. & Rodriguez-Schettino, L. (1998) Contingency and Determinism in Replicated Adaptive Radiations of Island Lizards. *Science*, **279**, 2115–2118.
- Mayr, E. (1963) *Animal Species and Evolution*. Harvard University Press, Cambridge, MA.
- Merriam, C.H. (1890) Results of a biological survey of the San Francisco Mountain region and desert of the Little Colorado in Arizona. *North American Fauna*, **3**, 1–136.
- Moore, S.W. (2003) Scrambled eggs: mechanical forces as ecological factors in early development. *Evolution and Development*, **5**, 61–66.
- Moran, N.A. (1992) The evolutionary maintenance of alternative phenotypes. *The American Naturalist*, **139**, 971–989.
- Narici, M.V., Roi, G.S., Landoni, L., Minetti, A.E. & Cerretelli, P. (1989) Changes in force, cross sectional area and neural activation during strength training and detraining of the human quadriceps. *European Journal of Applied Physiology*, **59**, 310–319.
- Padilla, D.K. & Adolph, S.C. (1996) Plastic inducible morphologies are not always adaptive: the importance of time delays in a stochastic environment. *Evolutionary Ecology*, **10**, 105–117.
- Pankakoski, E., Koivisto, I. & Hyvrinen, H. (1992) Reduced developmental stability as an indicator of heavy metal pollution in the common shrew, *Sorex araneus*. *Acta Zoologica Fennica*, **191**, 137–144.
- Parfitt, A.M. (1997) Genetic effects on bone mass and turnover-relevance to black/white differences. *Journal of the American College of Nutrition*, **16**, 325–333.
- Power, J., Loveridge, N., Rushton, N., Parker, M. & Reeve, J. (2002) Osteocyte density in aging subjects is enhanced in bone adjacent to remodeling haversian systems. *Bone*, **30**, 859–865.
- Robling, A.G., Castillo, A.B. & Turner, C.H. (2006) Biomechanical and molecular regulation of bone remodeling. *Annual Reviews of Biomedical Engineering*, **8**, 455–498.
- Rohlf, F.J. (2005) *tpsDig*, digitize landmarks and outlines version 2.05. Department of Ecology and Evolution, State University of New York, Stony Brook.
- Rohlf, F.J. & Slice, D. (1990) Extensions of the Procrustes method for the optimal superimposition of landmarks. *Systematic Zoology*, **39**, 40–59.
- Ruff, C. (2003) Growth in bone strength, body size, and muscle size in a juvenile longitudinal sample. *Bone*, **33**, 317–329.
- Ryan, A.S., Treuth, M.S., Rubin, M.A., Miller, J.P., Nicklas, B.J., Landis, D.M., Pratley, R.E., Libanati, C.R., Gundberg, C.M. & Hurley, B.F. (1994) Effects of strength training on bone mineral density: hormonal and bone turnover relationships. *Journal of Applied Physiology*, **77**, 1678–1684.
- Scheiner, S.M. (1993) Genetics and evolution of phenotypic plasticity. *Annual Review of Ecology and Systematics*, **24**, 35–68.
- Schlichting, C.D. & Pigliucci, M. (1998) *Phenotypic Evolution*. Sinauer Associates, Sunderland.
- Sharma, D.R. (1958) Studies on the Anatomy of the Indian Insectivore, *Suncus Murinus*. *Journal of Morphology*, **102**, 427–553.
- Simpson, G.G. (1953) *The Major Features of Evolution*. Columbia University Press, New York, NY.
- Skerry, T. (2000) Biomechanical influences on skeletal growth and development. *Development, Growth and Evolution: Implications for the Study of the Hominid Skeleton* (eds P. O'Higgins & M.J. Cohn), pp. 29–39. Academic Press, New York.
- Smith, M.M. & Hall, B.K. (1990) Development and evolutionary origins of vertebrate skeletogenic and odontogenic tissues. *Biological Reviews of the Cambridge Philosophical Society*, **65**, 277–374.
- Thomason, J.J. (1991) Cranial strength in relation to estimated biting forces in some mammals. *Canadian Journal of Zoology*, **69**, 2326–2333.
- Vermeij, G.J. (1973) Adaptation, versatility, and evolution. *Systematic Zoology*, **22**, 466–477.
- Wainwright, P.C., Alfaro, M.E., Bolnick, D.I. & Hulsey, C.D. (2005) Many-to-one mapping of form to function: a general principle in organismal design? *Integrative and Comparative Biology*, **45**, 256–262.
- West-Eberhard, M.J. (2003) *Developmental Plasticity and Evolution*. Oxford University Press, Oxford.
- Young, R.L. & Badyaev, A.V. (2007) Evolution of ontogeny: linking epigenetic remodeling and genetic adaptation in skeletal structures. *Integrative and Comparative Biology*, **47**, 234–244.
- Young, R.L., Haselkorn, T.S. & Badyaev, A.V. (2007) Functional equivalence of morphologies enables morphological and ecological diversity. *Evolution*, **61**, 2480–2492.
- Zakharov, V.M., Pankakoski, E., Sheftel, B.I., Prltonen, A. & Hanski, I. (1991) Developmental stability and population dynamics in the common shrew, *Sorex araneus*. *The American Naturalist*, **138**, 797–810.

Received 23 March 2009; accepted 20 October 2009

Handling Editor: Peter Wainwright