EVOLUTION AND DEVELOPMENT OF DIVERSITY: AN EXAMPLE IN FORAGING MORPHOLOGY OF SORICID SHREWS

by

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DEDICATION

To my parents, Kaye and Tom, for their ever-present love and unwavering support
# TABLE OF CONTENTS

ABSTRACT ........................................................................................................................7  
CHAPTER I: INTRODUCTION ........................................................................................9  
CHAPTER II: PRESENT STUDY ...................................................................................16  
REFERENCES .................................................................................................................19  
APPENDIX A. EVOLUTION OF ONTOGENY: LINKING EPIGENETIC REMODELING AND GENETIC ADAPTATION IN SKELETAL STRUCTURES .......................................................................................................26  
APPENDIX B. EVOLUTIONARY PERSISTENCE OF PHENOTYPIC INTEGRATION: INFLUENCE OF DEVELOPMENTAL AND FUNCTIONAL RELATIONSHIPS ON COMPLEX TRAIT EVOLUTION ..................................62  
APPENDIX C. FUNCTIONAL EQUIVALENCE OF MORPHOLOGIES ENABLES MORPHOLOGICAL AND ECOLOGICAL DIVERSITY ..............................93  
APPENDIX D. DEVELOPMENTAL PLASTICITY LINKS LOCAL ADAPTATION AND DIVERSIFICATION IN THE FORAGING MORPHOLOGY OF SHREWS .........................................................................................................135
ABSTRACT

Divergent natural selection for use of locally abundant resources can lead to diversification within and across species. However, the consequences of divergent selection for phenotypic evolution also depend on the development of variation. Because relationships among traits such as shared developmental timing or common involvement in an organismal function can channel variation generated during development, these relationships strongly influence the direction of evolution.

During development of the mammalian mandible multiple tissues of distinct developmental origins interact with inputs from the functioning of attached muscles to produce a cohesive and well integrated trait. In soricid shrews, part of the mandible matures late in ontogeny, coinciding with the onset of foraging. In this case, foraging-linked muscle activity should influence the development of the late maturing mandibular region. Here, I show that variation in this late ossifying region reveals the local functional requirements of the jaw and results in an opportunity to decouple internal and external sources of variation (developmental and environmental respectively) in the mandible. Capitalizing on this feature of the *Sorex* system, I empirically examined the historical persistence of internal and external patterns of variation, the consequences of variation patterning for ecological and morphological diversification across taxa, and differences between early and late ossifying regions in their contribution to local adaptation in mandible morphology.

I found that the functional requirements of diet directed mandible development and determined species similarity in both mandible morphology and function. Timing of
bone maturation determined the morphological effects of foraging-linked muscle activity, resulting in differential expression of adaptive variation in the late maturing region. Further, I found higher levels of interspecific variation in the late maturing region of the mandible, and showed that interspecific divergence in foraging morphology occurs along the lines delineated by epigenetic inputs of muscle on bone formation during late ontogeny within species. These findings indicate that differences in functional requirements are critical for divergence among taxa in this system. Further, these results suggest that, when external inputs into trait development are indicative of local functional requirements, the same epigenetic mechanism of development can generate diversity both within and among taxa.
CHAPTER I: INTRODUCTION

Explanation of the Problem and its Context

Morphological diversity is often attributed to distinct selection pressures across environments (Wainwright and Reilly 1994; Losos et al. 1998; Schluter 2000). However, evolution requires not only selection, but also the presence phenotypic variation. Thus, a complete understanding of adaptive evolution and diversification in morphological traits requires knowledge of the development origins of phenotypic variation as well as how patterns of developmental variation influence morphological response to selection (Waddington 1941; Mayr 1962; Alberch 1980; Schlichting and Pigliucci 1998; West-Eberhard 2003). Few studies to date provide both an examination of the developmental origins of morphological variation and an assessment of the functional and evolutionary consequences of the resulting morphology.

The revival of a developmental perspective in evolutionary biology has led to an increased understanding of how variation is generated and patterned during development (Hall 1992; Amundson 1994, 2005; Newman and Müller 2000, 2005; Arthur 2001; Salazar-Ciudad and Jernvall 2004) and a renewed focus on the role of the environmentally-induced variation in phenotypic evolution (Baldwin 1902; Waddington 1949; Schlichting and Pigliucci 1998; Gilbert 2001; West-Eberhard 2003; Schlichting 2004; Badyaev 2005b). However, evidence of a mechanistic link between developmental plasticity and the evolution of diversity in limited (Wright 1931; Simpson 1953; Schluter 2000; de Jong 2005; but see, Gomez-Mestre and Buchholz 2006).
Epigenetic regulation of skeletal development an opportunity to study evolutionary consequences of developmental plasticity, because ontogenetic variation in internal and external causes of epigenetic inputs and their corresponding effect on skeletal formation link directly developmental plasticity and timing of natural selection for local adaptation. During development of skeletal traits, a variety of inputs (e.g., tissue interactions, genes, and the environment) interact to produce a cohesive, well-integrated and functional trait (Wessells 1977; Hall 1984b; Atchley et al. 1985; Dammrich 1991; Herring 1993a). While the role of these interactions for normal development and function are well established, explicitly defining the importance of distinct sources of variation for phenotypic adaptation and diversification has been illusive.

This dissertation examines the development and evolution of diversity in foraging morphology of Sorex shrews. Shrews are an ecologically diverse group of mammals that utilize a variety of prey items, resulting in distinct functional requirements of the jaw across environments. In shrews, growth and maturation of part of the mandible is delayed and coincides temporally with initiation of foraging. Here, I show that response of the late maturing region of the mandible to functioning of attached muscles reveals environmental sources of variation in development (Appendix B: Young and Badyaev 2006; also see Appendix D). This characteristic of mandible development makes soricid shrews a powerful system to examine the role of environmentally-induced variation in morphological evolution. Here, I report findings of a series of studies that examine the consequences of environmentally-induced variation for ecological, morphological, and functional diversification across species of shrews (Appendices B and C). In addition, I
review and integrate concepts of evolutionary developmental biology and propose a mechanism linking the evolution of environmental sensitivity of development and the developmental incorporation of environmentally-induced phenotypes (Appendix A). Finally, I provide empirical evidence for the importance of the proposed mechanism for adaptation and diversification of the shrew mandible (Appendix D).

Review of the Literature

Causes and consequences of morphological integration—Morphological traits often consist of simpler units that are coordinated in development or function (Olson and Miller 1958; Cheverud 1984, 1996; Wagner 1996, 2001). The degree to which structures can be subdivided into tightly integrated semi-independent units or modules is crucial for morphological evolution and diversification (Lewontin 1974; Bonner 1988; Raff 1996; Kirschner and Gerhart 1998; Von Dassow and Munro 1999; West-Eberhard 2003). This organization of complex morphological structures allows for modification of individual units without disrupting overall functionality, and as a result can facilitate the evolution of ecological, functional, and morphological diversity (e.g., see Alfaro et al. 2005; Wainwright et al. 2005; Young et al. 2007). At the same time, strong, consistent covariation patterns may limit evolvability in certain directions (Vermeij 1970, 1973; Maynard Smith et al. 1985; Schwenk 1995; Schlichting and Pigliucci 1998; Arthur 2001).

Recent empirical and theoretical work suggests that the evolutionary consequences of integration and modularity depend on how trait covariation arises during ontogeny (Wimsatt 1994; Eble 2003; Schlosser and Wagner 2004). Two distinct
developmental mechanisms resulting in trait covariation have been described in detail, direct developmental interactions and parallel variation of distinct developmental pathways (Riska 1986; Wagner 2001; Klingenberg 2005). Covariation due to direct developmental connections results when traits share common developmental precursors, pathways, or resources (Riska 1986; Raff 1996; Klingenberg and McIntyre 1998; Hall 2003; Badyaev 2004; Young and Badyaev 2006). Because of the direct connection between developmental pathways of multiple traits, variation in development of one trait will necessarily result in correlated variation in the other traits regardless of the source of variation. Covariation among traits due to parallel variation of distinct developmental pathways, on the other hand, results when multiple traits respond to a common stimulus (e.g. genetic or environmental perturbations that affect multiple developmental pathways) (de Jong 1999; Sasaki and de Jong 1999; Schwenk 2001; Badyaev and Foresman 2004; Badyaev and Young 2004; Young and Badyaev 2006). In this case, covariation among traits is a direct result of the source of variation; thus, the absence of or a change in the particular stimulus will result in changes in covariation patterns.

Overall patterning of variation in morphological structures, or modularity, results from a combination of these two mechanisms, direct developmental connections and parallel variation of distinct developmental pathways. For example, in skeletal traits, components that arise from the same developmental precursors share direct developmental connections while components involved in attachment of the same muscle will respond similarly to strain applied by that muscle regardless of the developmental
relationships among them. Thus, the evolutionary consequences of trait covariation patterns depend on the relative importance of developmental mechanisms of integration.

Regulation of development in the mammalian mandible—The mammalian mandible has been widely studied as a model of evolution and development of complex structures (Hall 1980, 1984b; Zelditch 1988; Atchley and Hall 1991; Atchley 1993; Klingenberg et al. 2003). Extensive research on the developmental and genetic architecture of the mandible has described mandible formation in detail (Atchley and Hall 1991). Skeletal development is a dynamic process that requires activity of many genes regulating transition between growth and maturation of cells (Smith and Hall 1990; Atchley and Hall 1991; Atchley 1993; Thorogood 1993; Hogan 1996; Skerry 2000; Chen et al. 2004; Yoon and Lyons 2004; Tsumaki and Yoshikawa 2005; Wutzl et al. 2006). These genetic pathways are largely epigenetically regulated by external stresses (Herring 1993b; Huiskes 2000; Skerry 2000; Rauch and Schoenau 2001; Lobe et al. 2006). Variation in mechanical stress induces changes in cell signaling and gene expression leading to changes in rates of cell division, cell death, differentiation, and ossification (Skerry 2000; Moore 2003; Müller 2003; Noble et al. 2003; Segev et al. 2004; Zelditch 2005; Young and Badyaev 2007).

Both genetic and epigenetic variation in developmental processes results in modification of the mandible (Atchley and Hall 1991). However, the development of variation in mammalian craniomandibular structures has been attributed primarily to epigenetic interactions between soft (e.g. muscles) and hard tissues (Cheverud 1982;
Temporal and spatial distribution of mechanical stresses can channel developmental variation through differential growth (Henderson and Carter 2002; Badyaev 2005a; Badyaev et al. 2005; Zelditch 2005) and decoupling of ossification timing among units (Hall 1984a; Smith 2002; Young and Badyaev 2007). Thus, epigenetic inputs can illicit developmental responses important for the generation of morphological variation; however their specific role of in the evolution of diversity in the mammalian mandible is unclear.

Explanation of Dissertation Format

This research conceptually and methodologically integrates studies of development, phenotypic evolution, and functional morphology to elucidate how the developmental mechanisms that produce variation during organismal ontogeny ultimately generate interspecific diversity in animal form and function.

In Appendix A, I capitalize on existing knowledge of molecular and developmental features of bone formation. I conducted an extensive literature review of empirical and experimental studies examining genetic and epigenetic regulation of skeletal development, specifically focusing on the role of bone morphogenetic proteins (BMPs). I conceptually integrate this knowledge of genetics and development of bone formation with fundamental concepts of evolutionary developmental biology: heterochrony, developmental plasticity, phenotypic accommodation, and genetic assimilation, to propose a novel hypothesis linking the evolution of developmental plasticity and the developmental incorporation of environmentally-induced phenotypes.
In Appendix B, taking advantage of developmental characteristics of the shrew mandible, I first present a method for decoupling patterns of developmental and functional integration. Second, using methods of comparative biology, I examine the consequences of integration for mandible evolution by describing pattern of historical persistence of both developmental and functional integration across nine species of shrews.

In Appendix C, I examine the consequences of complexity (i.e., modularity) for the evolution of diversity in shrews. I characterize species ecology by compiling and analyzing published data on species diet type, foraging preferences, and habitat preferences in 15 *Sorex* species. Next, I assess functional variation among taxa using a previously established model of bite force in the shrew mandible. Using a comparative approach, I examine how the modular structure of the mandible influences functional and morphological adaptation to distinct ecological niches across taxa. Finally, I developed simulations to define the limits of diversification in mandible morphology.

In Appendix D, I combined detailed empirical data on 1) the developmental effects of foraging-linked muscle activity on the morphology of the early and late maturing regions of the mandible, 2) empirical measurements of mandible performance (i.e., bite force), and 3) an examination of the degree and direction of interspecific variation in the early and late ossifying regions of the mandible to test whether the same epigenetic mechanism – the developmental effects of muscle activity – can generate both adaptation within species and diversity among species.
CHAPTER II: PRESENT STUDY

The methods, results, and conclusions of this study are presented in the papers appended to this dissertation. The following is a summary of the most important findings of these papers.

To develop a more complete understanding of the role of developmental plasticity in diversification of skeletal traits, I combined a synthesis of development and evolution of diversification in skeletal traits with a detailed empirical evaluation of the importance of environmental sensitivity of development for adaptation and diversification of morphology and function of the soricid shrew mandible. In Appendix A, I integrate knowledge of genetics, development, and evolution of bone formation and present a novel hypothesis of adaptation and diversification of skeletal structures. I show that timing of maturation in skeletal traits should play a critical role in both phenotypic accommodation of environmentally induced developmental pathways and flexibility in development across environments. I suggest that heterochronic shifts in timing of ossification relative to exposure to unpredictable environments can not only buffer development under fluctuating environments while maintaining epigenetic sensitivity critical for normal skeletal formation, but also enable epigenetically induced gene expression to generate specialized morphological adaptations.

In Appendix B, I compare historical persistence of integration patterns produced by developmental relationships versus patterns of integration generated by functional requirements of the mandible by examining mandible evolution in nine species of soricid shrews. I show that, irrespective of phylogenetic relatedness between species, patterns of
developmental and functional integration were highly concordant. These findings suggest that the same developmental pathways channel both functional and developmental variation. This type of 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. Further, I found that divergence in mandible shape among species closely follows variation in functional demands and ecological requirements regardless of phylogenetic relatedness among species.

In Appendix C, I examine the factors limiting variation in mandible morphology across shrew species by assessing the relative contribution of trait function (biomechanics of the jaw), ecology, and phylogeny to species similarity in mandibular traits. I show that the presence of multiple semi-independently varying traits, or modules, enabled functional equivalence of among distinct composite mandible morphologies. Moreover, the modular structure of the mandible enabled the evolutionary persistence of differences in habitat use (e.g., habitat moisture and coverage) among species that specialize on the same diet. Finally, I found that later ossifying tissues were the most variable across diet types suggesting that timing of ossification may be important in determining correlation structure among mandible components. In addition, this finding suggests that environmental sensitivity of development in these tissues may facilitate ecological diversification across shrew taxa.

In Appendix D, I examine the role of epigenetic regulation of mandible development for adaptation within taxa and diversification across taxa. In vertebrates, growth and development of bones is partially guided by contractions of the attached
musculature and such muscle activity changes progressively from sporadic early embryonic motility to directed functional effects at late embryonic stages. In species with short generation times, delayed skull maturation extends the directing effects of muscle activity on formation of foraging morphology into early adulthood, providing a unique opportunity to directly examine the links between plasticity of bone development, ecological adaptations, and evolutionary diversification in foraging morphology. I found that the late maturing elements of the mandible were more affected by muscle activity associated with the local functional requirements of foraging. I found that variation associated with individual bite force was limited to the late ossifying region of the mandible. Moreover, I found that the direction of interspecific variation in morphology of the late maturing region of the mandible was highly concordant with the direction of muscle-induced variation within species. These results highlight the importance of environmental sensitivity of development for diversification and suggest that, when external inputs into trait development are indicative of local functional requirements, the same epigenetic mechanism of development can generate diversity within and across taxa.
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APPENDIX A: EVOLUTION OF ONTOGENY: LINKING EPIGENETIC REMODELING AND GENETIC ADAPTATION IN SKELETAL STRUCTURES

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CONTENTS

Ecological Dimorphisms in Vertebrates: Proximate and Ultimate Causes
Organized by Shawn Vincent, Simon P. Lalvaox, Anthony Herrel, and Emily Taylor

169 Ecological dimorphisms: An introduction to the symposium
Simon P. Lalvaox and Shawn E. Vincent

172 Functional and ecological correlates of ecologically-based dimorphisms in squamate reptiles
Shawn E. Vincent and Anthony Herrel

109 Interactive effects of sex and temperature on locomotion in reptiles
Simon P. Lalvaox

200 Determinants of sexual differences in escape behavior in lizards of the genus Anolis: a comparative approach
Bishe Vanhooydonck, Anthony Herrel, and Duncan J. Irshick

211 Morphology, performance, behavior and ecology of three color morphs in males of the lizard Anolis carolinensis
Kathleen Hygh, Bishe Vanhooydonck, Anthony Herrel, Zoran Tadić, and Raoul Van Damme

221 Developmental evolution of sexual ornamentation: model and a test of feather growth and pigmentation
Alexander V. Badysé and Elizabeth A. Landeen

234 Evolution of ontogeny: linking epigenetic remodeling and genetic adaptation in skeletal structures
Rebecca L. Young and Alexander V. Badysé

245 Sexual segregation in vertebrates: proximate and ultimate causes
K. E. Ruckebshl

250 Proximate developmental mediators of sexual dimorphism in size: case studies from squamate reptiles
Henry B. John-Alder, Robert M. Cox, and Emily N. Taylor

272 Vive le différent! Sexual dimorphism and adaptive patterns in lizards of the genus Anolis
Marguerite A. Butler

285 Are powerful females powerful enough? Acceleration in gravid green iguanas (Iguana iguana)
Jeffrey Scales and Marguerite Butler

Ecology and Evolution of Disease Dynamics

295 Parasite castration: a perspective from a model of dynamic energy budgets
Spencer R. Hall, Class Becker, and Carla E. Caceres

310 Host resource supplies influence the dynamics and outcome of infectious disease
Val Smith

317 Why don’t all whales have cancer? A novel hypothesis resolving Peto’s paradox
John D. Nagy, Eric M. Victor, and Jonene H. Cropper
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ABSTRACT

Evolutionary diversifications are commonly attributed to the continued modifications of a conserved genetic toolkit of developmental pathways, such that complexity and convergence in organismal forms are assumed to be due to similarity in genetic mechanisms or environmental conditions. This approach, however, confounds the causes of organismal development with the causes of organismal differences and, as such, has only limited utility for addressing the cause of evolutionary change. Molecular mechanisms that are closely involved in both developmental response to environmental signals and major evolutionary innovations and diversifications are uniquely suited to bridge this gap by connecting explicitly the causes of within-generation variation with the causes of divergence of taxa. Developmental pathways of bone formation and a common role for bone morphogenetic proteins (BMPs) in both epigenetic bone remodeling and the evolution of major adaptive diversifications provide such opportunity. We show that variation in timing of ossification can result in similar phenotypic patterns through epigenetically induced changes in gene expression and propose that both genetic accommodation of environmentally induced developmental pathways and flexibility in development across environments evolve through heterochronic shifts in bone maturation relative to exposure to unpredictable environments. We suggest that such heterochronic shifts in ossification can not only buffer development under fluctuating environments while maintaining epigenetic sensitivity critical for normal skeletal formation, but also enable epigenetically induced gene expression to generate specialized morphological adaptations. We review studies of environmental sensitivity of BMP pathways and their
regulation of formation, remodeling, and repair of cartilage and bone to examine the hypothesis that BMP-mediated skeletal adaptations are facilitated by evolved reactivity of BMPs to external signals. Surprisingly, no empirical study to date has identified the molecular mechanism behind developmental plasticity in skeletal traits. We outline a conceptual framework for future studies that focus on mediation of phenotypic plasticity in skeletal development by the patterns of BMP expression.
RECONCILING PHENOTYPIC PATTERNS AND MOLECULAR MECHANISMS OF ADAPTATION

A goal of evolutionary biology is to understand the origins of diversity. Phenotypic diversity is thought to reflect extensive genetic variation, even among closely related taxa (Lauder 1981; Raff 1996; Carroll 2002). Because diversification often shows rapid and punctuated evolutionary patterns (Eldredge and Gould 1972; Gould and Eldredge 1993; Raff 1996), and because genes and developmental pathways are often broadly conserved, current hypothetical mechanisms for the origin and evolution of diversity invoke modifications of existing genetic networks rather than the evolution of novel genes or genetic pathways (Scott 1994; Gerhart and Kirschner 1997; Carroll 2001; Davidson 2001; Carroll 2002; Wilkins 2002; West-Eberhard 2003; Amundson 2005). Whereas this new focus advances our understanding of the mechanisms underlying the development and evolution of diversity, it is unclear how existing genetic networks can be modulated for a variety of developmental roles and contexts.

One hypothesis suggests that evolutionary change is facilitated by environmental sensitivity and by modifications of organismal development that induce selectable phenotypic variation (Fig. 1A and D). Moreover, previously neutral genetic variation may gain function under novel or stressful conditions, either through expression of allelic variation (Bergman and Siegal 2003; Hermitsson and Wagner 2004; Rice 2004; Wagner and Mezey 2004; Badyaev 2005b; Larsen 2005) or through exploitation of ectopically expressed gene products with no known function (reviewed in Rodriguez-Trelles 2004;
Rodríguez-Trelles et al. 2005; Yanai et al. 2006). Environmentally induced recruitment of this “hidden” variation may facilitate the generation of new and favored phenotypic variants through developmental changes induced by novel patterns of gene expression (Fig.1A–D).

Thus, the environment can facilitate generation of morphological variants either by inducing developmental plasticity or by challenging organismal development, exposing previously unexpressed genetic variation (Fig. 1A). At the same time, the environment also selects induced phenotypes (Fig. 1E), and such selection can act on the developmental mechanism producing that phenotype (Fig. 1G). The long-term consequences of selection on environmentally induced phenotypes depend on the within-generation reliability (e.g., similarity between the signaling and selecting environments) and across-generation predictability of the environment (Oyama 2000; West-Eberhard 2003). If the inducing environment and the selection on the induced phenotype are predictable, then selection should favor greater developmental sensitivity of phenotypes to the inducing environment (Schlichting and Pigliucci 1998; West-Eberhard 2003; Pigliucci et al. 2006). Over time, repeatability and predictability of an external signal should favor the developmental incorporation of the environmentally induced developmental pathways by favoring genotypes that reliably develop a consistent phenotype across generations (i.e., genetic accommodation; Baldwin 1896; Schmalhausen 1949; Schlichting and Pigliucci 1998; West-Eberhard 2003; Pigliucci et al. 2006).
Whereas the predictions of this hypothesis are consistent with the historical patterns of diversification (reviewed in West-Eberhard 2003), it is unclear how the developmental origins of adaptation and diversification can be integrated with the presumed modulation of existing genetic networks as a mechanistic basis for the evolution of diversity. Illustration of such integration requires a system in which the genetic network regulating adaptive diversification among taxa also mediates within-taxon developmental plasticity. Here, we suggest that genetic pathways of skeletal development fulfill this role because of their common involvement in both epigenetic regulation of growth and remodeling of cartilage and bone in response to mechanical stress as well as development of highly specialized morphological adaptations and innovations (Table 1). Thus, examination of this system provides a unique opportunity to unify the developmental origin of adaptation hypothesis with the proposed molecular mechanism of the development and evolution of diversity.

A CASE STUDY IN SKELETAL DEVELOPMENT AND ADAPTATION

Growth and development of skeletal structures involves a series of transitions between cell proliferation and differentiation (e.g., transitions between cartilage and bone) largely regulated by expression of bone morphogenetic proteins (BMPs) (Hogan 1996; Urist 1997; Chen et al. 2004; Tsumaki and Yoshikawa 2005). Variation in timing and spatial organization of these transitions in cell function is critical in the development of diverse phenotypes; recent studies of evolutionary innovations and adaptive radiations
in vertebrate morphology have implicated variations in BMP expression as primary mechanisms inducing adaptive developmental changes in cartilage and bone (Table 1). Changes in patterns of BMP expression typical of skeletal adaptations (Table 1) are frequently hypothesized to result from mutations in regulatory regions of BMP pathways (Terai et al. 2002; Albertson and Kocher 2006). However, this hypothesis overlooks the crucial role of environmental and other non-genetic inputs into skeletal development despite overwhelming evidence of the close relationship between external stimuli (e.g., muscle loading and diet) and the development of cartilage and bone (Herring 1993; Huiskes 2000; Rauch and Schoenau 2001; Moore 2003; Müller 2003; Lobe et al. 2006).

Bone formation is a dynamic process that involves activity of many genes regulating transitions between growth and maturation of cells (Smith and Hall 1990; Atchley and Hall 1991; Atchley 1993; Hogan 1996; Skerry 2000; Chen et al. 2004; Yoon and Lyons 2004; Tsumaki and Yoshikawa 2005; Wutzl et al. 2006). Importantly, these complex genetic pathways of growth, maturation, and remodeling of cartilage and bone are largely regulated by external stress (Herring 1993; Huiskes 2000; Skerry 2000; Rauch and Schoenau 2001; Moore 2003; Müller 2003; Lobe et al. 2006). In fact, much of the variation in skeletal structures is attributed to both internal and external stresses inducing growth and differentiation (Frost 1987; Huiskes 2000; Rauch and Schoenau 2001; Mao and Nah 2004; Badyaev and Foresman 2004; Badyaev et al. 2005; Archer et al. 2006). Such sensitivity to stresses might reflect the importance of internal mechanical stresses for achieving close functional integration between soft (e.g., muscles or blood vessels)

Here, we review the role of epigenetic regulation of cartilage and bone formation, remodeling and repair for the evolution of diversity and adaptation in skeletal structures. First, we examine factors that influence patterns of gene expression, including both epigenetic and genetic effects, and discuss their importance for morphological evolution. Second, we establish the importance of external stimuli for prenatal and postnatal gene expression patterns, and provide evidence for the existence of individual variation in environmental sensitivity of genetic pathways. Third, we discuss the importance of environmental predictability for the evolution of induced phenotypes. Finally, we propose a hypothesis that a shift in timing of development provides a mechanism enabling not only developmental incorporation of environmentally induced phenotypes across generations, but also increased environmental sensitivity of trait development to epigenetic or environmental stimuli.

VARIATION IN GENE EXPRESSION

Variation in gene expression in skeletal development can result from several factors. First, external stresses on developing tissues can initiate changes in gene expression via modification of the cellular and intercellular environments (Table 2; Skerry 2000; Rauch and Schoenau 2001; Moore 2003). Second, genetic variation resulting from mutations in regulatory regions can modify timing, location, or levels of gene expression (Terai et al. 2002). Lastly, neutral genetic variation (e.g., “hidden” allelic
variation or ectopic expression) can result from either neutral, or unexpressed, variation in regulatory genes or from neutral variation in gene expression due to the complexity of regulatory networks (reviewed by Rodríguez-Trelles et al. 2005). This previously “hidden” variation can facilitate the development of phenotypic variants that might be adaptive under novel environments (Rodríguez-Trelles et al. 2005).

**Epigenetic effects: an example with BMPs**

Mechanical stresses are crucially important for regulation of chondrogenesis and osteogenesis, as well as bone remodeling and repair (Herring and Lakars 1981; Lanyon 1984; Frost 1987; Atchley et al. 1991; Herring 1993; Thorogood 1993; Huiskes 2000; Skerry 2000; Rauch and Schoenau 2001; Mao and Nah 2004). Experimental studies have implicated BMP-2, BMP-3, BMP-4, and, to a lesser extent, BMP-6 as well as Ihh and FGF-2 (known to regulate BMP expression or function) as critically important for the incorporation of external stimuli during skeletal development (Table 2). Moreover, these studies show that timing of mechanical stimulation influences the developmental response to the stimulus. During chondrogenesis BMP-2, BMP-4, and FGF-2 were upregulated under mechanical stress (Wu et al. 2001; Fong et al. 2003) and stress-induced upregulation of these genes determined the transition between growth and differentiation of chondrocytes (Pizette and Niswander 2000; Warren et al. 2003; Goldring et al. 2006). Similarly, when mechanical stimulation was applied during bone growth, BMP-4 was upregulated resulting in increased proliferation and differentiation of osteoblasts (Ikegame et al. 2001; Wu et al. 2001). At the same time, BMP-3 was
markedly downregulated during experimentally stimulated bone remodeling, inhibiting bone formation and enabling cartilage differentiation (Aspenberg et al. 2000; Hino et al. 2004). Finally, when bones were experimentally damaged by artificial fractures or surgical separation, BMP-2, BMP-4, and BMP-6 were upregulated inducing growth and subsequent ossification of cartilage (Nakase et al. 1994; Sato et al. 1999; Tsuji et al. 2006).

The effects of mechanically induced expression of BMPs (especially BMP-2 and 4) on growth and development also varied with intensity and duration of mechanical stimulation (Sato et al. 1999; Wu et al. 2001; Mao and Nah 2004). For example, in cranial suture zones, upregulation of BMP-4 initially resulted in growth of osteoblasts, but under prolonged stress led to their maturation (Ikegame et al. 2001; Wu et al. 2001). This variation in phenotypic response to changes in BMP expression likely reflects dose-dependency of the effects of BMPs—a common finding in studies of regulatory networks (Hogan 1996; Davidson 2001; Mao and Nah 2004). The wide spectrum of changes in gene expression patterns that can be induced by mechanical stimulation suggests that such induction has significant evolutionary potential. Indeed, differences in timing of BMP expression are crucial for several adaptive radiations in vertebrate morphologies (Table 1).

Genetic effects

In addition to epigenetic regulation, mutations in regulatory, promoting, and processing regions of genetic pathways of bone formation can generate changes in gene
expression. In particular, mutations in regulatory or processing regions allow for changes in gene expression without disrupting cohesiveness of developmental networks (Davidson 2001), and the complexity of regulatory networks represents large mutational targets (Stern 2000; Carroll et al. 2001, Siegal and Bergman 2002). Increased generation of phenotypic variation under this scenario should facilitate diversification of regulatory pathways and corresponding skeletal structures. For example, in a broad examination of molecular evolution in morphogenetic genes among cichlids, Terai et al. (2002) found allelic variation in the prodomain of BMP-4 consistent with high levels of morphological variation; this variation was related to changes in protein folding, and thus modified downstream effects without disrupting the general function of the gene (Bryan 2002; Terai et al. 2002). However, while fortuitous mutations in regulatory regions of BMPs may facilitate adaptation in some systems, it is unlikely to be the main reason for BMP ubiquity in morphological adaptation and innovation because the lag time required for fixation of a favorable mutation far exceeds the rapid appearance of several BMP-mediated innovations (Table 1).

“Hidden” genetic effects

Patterns of gene expression are often assumed to be confined to times, locations, and levels appropriate to their specific function (Emerson 2003); however, recent studies have revealed high variability in gene expression patterns (reviewed by Rodríguez-Trelles 2004; Rodríguez-Trelles et al. 2005; Yanai et al. 2006). In novel environments, recruitment of these “hidden” gene products may facilitate development of new
phenotypic variants (Rodríguez-Trelles et al. 2005), and developmental exposure of “hidden” allelic variation under stress is often documented (Bergman and Siegal 2003; Hermisson and Wagner 2004; Rice 2004; Wagner and Mezey 2004; Badyaev 2005b; Larsen 2005). Alternatively, variation in gene expression can be produced by “expression leakage,” when functional expression of one gene results in nonfunctional expression of neighboring genes (e.g., transcriptional read-through; Rodríguez-Trelles et al. 2005; Yanai et al. 2006). Exposure of “hidden” variation in gene expression may be especially important in the origin of novel traits because it can induce novelty in the absence of pre-existing functional gene expression (Schlichting and Pigliucci 1998; Newman and Müller 2001; Yampolsky and Stoltzfus 2001; Schlichting 2003; Newman and Müller 2005; Rodríguez-Trelles et al. 2005).

**EVOLUTIONARY CONSEQUENCES: GENETIC ACCOMMODATION OR ENVIRONMENTAL SENSITIVITY?**

Novel environments can induce developmental changes by either epigenetic induction of variation in gene expression or by recruitment of existing variation in neutral allelic or ectopic expression (reviewed in Badyaev 2005a,b; Rodríguez-Trelles et al. 2005). While the production of phenotypic variation via plasticity development and remodeling of bone is common, the mechanisms by which environmentally induced changes in gene expression can generate evolved and specialized morphological adaptations (Table 1) are poorly understood. Here we discuss inheritance of
environmentally induced phenotypes and evolutionary incorporation of epigenetic signals into the normal developmental repertoire in relation to predictability of the inducing environment and selection.

**Inheritance of environmentally induced phenotypes**

Inheritance of environmentally induced phenotypes requires that individuals differ in sensitivity, exposure, or response to environmental signals and that these differences have a genetic component (Scheiner 1993; West-Eberhard 2003; Pigliucci et al. 2006). The first line of evidence for genetic underpinnings of environmentally induced phenotypes comes from studies of the genetics of phenotypic plasticity. The ubiquity of gene-by-environment interactions in quantitative genetics studies of phenotypic variation suggests that genetic variation in plasticity is abundant in nature and is not limited to the accumulation of neutral variation described earlier (for examples in skeletal traits see Heaney 1995; Parfitt 1997; reviewed in Scheiner 2002; Pigliucci 2005). Genetic canalization, common in complex genetic networks, can buffer organismal development from mutations; however, this canalization can break down under novel or stressful environmental conditions resulting in variable gene expression and facilitating the appearance and inheritance of induced phenotypes (reviewed in Badyaev 2005a,b). At the same time, accumulation and occurrence of both genotypic variation in plasticity and “hidden” genetic variation depends on environmental variability over time, and is thus determined by a population’s evolutionary history (Meyers 2005; Rapp and Wendel 2005). However, the requirements for maintenance of genetic variation under these two
scenarios differ, whereas genetic variation in developmental plasticity is maintained by fluctuating selection (de Jong 1999; de Jong and Gavrilets 2000), neutral variation is accumulated over time (Hermisson and Wagner 2004). When environmental change reveals neutral genetic variation, this previously “hidden” variation is exposed to selection resulting either in fixation or loss. Therefore, fluctuating environments that facilitate the accumulation of genetic variation in plasticity reduce levels of neutral genetic variation.

*Predictability of external signals and the evolution of environmentally induced traits*

The evolutionary consequences of selection on phenotypic variants depend on the reliability of external signals within a generation (Figs. 1E and 2), the predictability of the environment across generations (Fig. 2), and the source of induced variation. On the one hand, environmentally induced phenotypes resulting from exposure of “hidden” genetic variation under novel environmental conditions should lead to rapid accommodation of phenotypes through loss or fixation of previously neutral genetic variation. On the other hand, the evolutionary consequences of selection for phenotypic variants generated by developmental plasticity depend on the trans-generational predictability and the within-generational reliability of the environmental signals (Fig. 2; West-Eberhard 2003; Gluckman et al. 2007). If an environment is reliable within and across generations, then selection should predictably favor the same phenotype (Fig. 2A). Thus, genotypes consistently associated with a particular phenotype should be favored, resulting in a reduction in environmental sensitivity of trait development via genetic accommodation.
(Fig. 3). Similarly, if the environment is variable within, but predictable across, generations (Fig. 2B), then over time, selection should again favor the same phenotype. If, however, the environment is variable across generations but constant within a generation (e.g., in short-lived species, Young and Badyaev 2006; Young RL, Haselkorn TS, Badyaev AV, unpublished data) (Fig. 2C), then selection should favor the evolution of environmental sensitivity in trait development (e.g., longer overlap of trait development and function, Fig. 2C), ultimately producing high within-generation phenotypic variability (Fig. 3). Finally, if the environment is variable both within and across generations, then selection should favor the evolution of within-generational flexibility (Fig. 2D; e.g., high rates of remodeling bone) allowing adjustment to shifting phenotypic optima throughout an organism’s lifetime (Piersma and Drent 2003).

**SKELETAL ADAPTATIONS—WHAT IS EVOLVING?**

We have shown that phenotypic variation generated by environmentally induced changes in gene expression can be inherited either via the evolution of developmental plasticity or exposure of previously “hidden” genetic variation, and that the evolutionary consequences of selection on environmentally induced phenotypes should result in either genetic accommodation, generating consistent phenotypes across environments, or the evolution of greater environmental sensitivity of development, generating high levels of phenotypic variation in each generation (Figs. 2 and 3). However, the mechanisms by which these two distinct outcomes occur remain unclear.
Given the requirement of epigenetic regulation for normal bone formation (Warrell and Taylor 1979; Lanyon 1984; Herring 1993; Thorogood 1993), any developmental change leading to loss of responsiveness to mechanical or other epigenetic signals would be detrimental. Instead, developmental incorporation of previously environmentally induced pathways and retention of sensitivity to internal inputs can be accomplished by shifts in the relative timing of development and environmental exposure (Fig. 4A). Exposure to unpredictable environmental signals commonly increases throughout ontogeny, and as organisms approach maturity and bones ossify, sensitivity of development of the trait to epigenetic signals decreases (Fig. 4A). Evolutionary shifts in timing of development in relation to organismal exposure to unpredictable environments (Fig. 4) should allow for either developmental accommodation of induced pathways or the evolution of developmental plasticity without disrupting overall epigenetic regulation of skeletal development. Under this scenario, evolutionary incorporation of induced phenotypes can result either from earlier maturation of skeletal morphologies (Fig. 4B1), or by delaying organismal exposure to the environment (Fig. 4B2), e.g., longer gestation or time until dispersal from nest. Reduced exposure of trait development to unpredictable signals should limit the diversity of induced phenotypes, thus facilitating reliable development of a particular, favored morphology (Fig. 5C; Young RL, Haselkorn TS, Badyaev AV, unpublished data). Alternatively, the evolution of developmental plasticity might result from delay in maturation (Fig. 4C1 and C2). In this case, phenotypic accommodation of external stimuli experienced early in development should enable diversity in developmental response facilitating development of locally appropriate
morphologies (Fig. 5B). These heterochronic shifts in development of skeletal traits in relation to exposure to unpredictable environments are consistent with observed variation generated by environmentally induced changes in gene expression; earlier or increased expression of Ihh, BMP-2, or BMP-4 can result in premature ossification, thereby inhibiting developmental response to environmental variation (Table 2). Alternatively, delayed ossification may reflect upregulation of FGF-2, prolonging exposure to epigenetic signals (Table 2). Indeed, molecular mechanisms underlying many ecomorphological skeletal phenotypes involve heterochronic shifts in the BMP expression patterns (Table 1).

CONCLUSIONS

Drawing upon concepts of evolutionary developmental biology, we show that examination of developmental pathways of bone formation provides a unique opportunity to reconcile phenotypic patterns and molecular mechanisms of morphological evolution. We suggest that both genetic accommodation of environmentally induced developmental pathways and flexibility in development across environments evolves through heterochronic shifts in bone maturation relative to exposure to unpredictable environments. Furthermore, variation in timing of developmental events, such as ossification, can result in similar phenotypic patterns through epigenetically induced changes in gene expression. Finally, we suggest that patterns of BMP expression generating phenotypic variation found in studies of morphological adaptation (Table 1) are consistent with this hypothesis. Whereas multiple morphological adaptations have
been attributed to changes in expression of BMPs, the proposed hypothesis suggests that increased phenotypic plasticity in skeletal development should be likewise mediated by patterns of BMP expression. Yet, to the best of our knowledge, no empirical study to date has identified the molecular mechanism behind developmental plasticity in skeletal traits. The approaches outlined here can provide conceptual framework for such future studies by explicitly linking the mediation of phenotypic plasticity in skeletal development to the patterns of BMP expression.

ACKNOWLEDGMENTS

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Table 1. Innovation and adaptation of skeletal structures associated with expression of BMPs.

<table>
<thead>
<tr>
<th>Character</th>
<th>Role of BMPs</th>
<th>References</th>
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<tbody>
<tr>
<td>Morphological innovation</td>
<td></td>
<td></td>
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<tr>
<td>turtle carapace</td>
<td>Development of dermal bone, or formation of the plate of the turtle shell, is induced by BMP and/or regulators of BMPs (e.g., Ihh) likely secreted by the developing ribs.</td>
<td>(Cebra-Thomas et al. 2005)</td>
</tr>
<tr>
<td>bat wing</td>
<td>BMP-2 expression is increased in the bat forearm. Elongation of wing digits in bats results from change in relative growth and differentiation in cartilage, both processes are likely to be regulated by BMPs.</td>
<td>(Sears et al. 2006)</td>
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<td>Adaptation of existing skeletal structures</td>
<td></td>
<td></td>
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<tr>
<td>cichlid jaws</td>
<td>Difference in jaw morphology between biting and sucking morphs is associated with levels of BMP-4 expression early in development. Similar morphological patterns have been experimentally induced via upregulation of BMP-4 in zebra fish.</td>
<td>(Terai et al. 2002; Albertson et al. 2005; Albertson and Kocher 2006)</td>
</tr>
<tr>
<td>bird bills</td>
<td>In Darwin’s finches and ducks, breadth and depth of the bill is associated with earlier and higher levels of BMP-4 expression. Similar phenotypes have been experimentally induced in chickens and zebra finches.</td>
<td>(Abzhanov et al. 2004; Wu et al. 2004, 2006)</td>
</tr>
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</table>
Table 2. Evidence for sensitivity of genetic pathways of bone formation to epigenetic signals.

<table>
<thead>
<tr>
<th>Gene</th>
<th>Description of effect</th>
<th>References</th>
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<tbody>
<tr>
<td>Growth</td>
<td></td>
<td></td>
</tr>
<tr>
<td>BMP-4</td>
<td>Tensile stress induced sustained upregulation of BMP-4 in mouse cranial suture zones.</td>
<td>(Ikegame et al. 2001; Mao and Nah 2004)</td>
</tr>
<tr>
<td>Ihh and BMP-2 and 4</td>
<td>Mechanical stretching resulted in upregulation of BMP-2 and 4. This response depended in part on the upregulation of Ihh (a primary regulator of BMPs) under mechanical stress.</td>
<td>(Wu et al. 2001; Mao and Nah 2004)</td>
</tr>
<tr>
<td>FGF-2</td>
<td>Tensile strain on cranial (calvarial) osteoblasts induced upregulation of FGF-2. As part of the BMP regulatory network, FGF-2 products compete with BMPs for receptors.</td>
<td>(Fong et al. 2003; Warren et al. 2003)</td>
</tr>
<tr>
<td>Remodeling</td>
<td></td>
<td></td>
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<tr>
<td>BMP 3</td>
<td>Tension applied to leg bones of rats resulted in downregulation of BMP-3.</td>
<td>(Aspenberg et al. 2000)</td>
</tr>
<tr>
<td>Repair</td>
<td></td>
<td></td>
</tr>
<tr>
<td>BMP-2, 4, and 6</td>
<td>Experimental lengthening of rat limbs by surgical breaking, separation, and induction of bone formation via application of mechanical stress resulted in increased expression of BMP-2 and BMP-4. BMP-6 also showed elevated levels later in bone formation.</td>
<td>(Sato et al. 1999)</td>
</tr>
<tr>
<td>BMP-4</td>
<td>Upregulation of BMP-4 in tissues surrounding an artificial fracture.</td>
<td>(Nakase et al. 1994)</td>
</tr>
</tbody>
</table>
Figure 1. Conceptual outline of the generation of phenotypic variants by changes in development. Development is influenced by a combination of epigenetic (A) and genetic (B) effects. (C) Sensitivity and response of development to epigenetic stimuli depend on both the strength of the stimulus and individual sensitivity to the signal. (D) Development (e.g., patterns of gene expression) determines the phenotypic outcome. (E) Environment provides the stimulus inducing change in development as well as the selection on the developing phenotype. Selection acts on both (F) the phenotype and (G) its development.
A - duration of trait development
   - duration of trait function
   - consistency of selection

B

C

D

Generations
Figure 2. Conceptual illustration of overlap between trait development and environmental exposure (e.g., through trait function) under distinct scenarios of within-generation reliability and trans-generational predictability of selection. (A) Selection is consistent within and across generations favoring a predictable phenotype and thus a decrease in exposure of development to unpredictable environments. (B) Selection is variable within a generation but predictable across generations, again, consistently favoring the same phenotype, and thus less overlap between trait development and function. (C) Selection is consistent within generations, but variable across generations. In this case, increase in overlap of development and function enables the development of locally appropriate phenotypes. (D) Selection fluctuates unpredictably within and across generations, favoring within-generation flexibility of phenotypes, and thus complete overlap of trait development and function. Gray lines indicate duration of development, black lines indicate duration of function, patterned bars illustrate selection (changes in patterning indicate differences in selection), and dashed gray lines separate generations.
Figure 3. Conceptual outline of evolutionary change in environmental sensitivity of development. (A) Reliability of the environment within and across generations determines the predictability of selection. (B) Predictability of selection determines the adaptive sensitivity to environmental variation during development by reliably favoring development of (C) a consistent phenotype or variable phenotypes.
Figure 4. Mechanism for evolutionary change in environmentally induced phenotypes.

(A) Throughout ontogeny, sensitivity of development to environmental signal decreases (black line), whereas exposure to unpredictable environmental signals increases (gray line). (B) Increased developmental buffering can result from heterochronic shifts resulting in (1) earlier maturation or (2) delayed exposure to the unreliable environmental signals. (C) Alternatively, increased developmental flexibility can result from (1) delays in maturation of a trait or (2) organismal exposure to environmental stimuli at earlier stages of development facilitating generation of phenotypic variation via developmental plasticity. Arrows illustrate direction of heterochronic shifts increasing (B) developmental buffering or (C) flexibility. Curved lines show relationship between developmental sensitivity and exposure after heterochronic shift.
Figure 5. Illustration of the influence of ontogenetic stage on response to external stimuli in the soricid shrew mandible. (A) Mandible tissues vary in ossification sequence; dark shading indicates earlier ossification. (B) External stress at early stages of ossification generates novel and variable phenotypes. (C) External stress at later stages of ossification results in channeled developmental variation resulting in similar patterns of variation among resulting phenotypes.
APPENDIX B: EVOLUTIONARY PERSISTENCE OF PHENOTYPIC INTEGRATION: INFLUENCE OF DEVELOPMENTAL AND FUNCTIONAL RELATIONSHIPS ON COMPLEX TRAIT EVOLUTION

Published: *Evolution* (2006) 60: 1291-1299
CONTENTS

(Continued from front cover)

Phylogenetic Analysis of the Cardini Group of Drosophila with Respect to Changes in Pigmentation • Jennifer A. Beisson, Jason Wilder, and Hope Hollocher ................................................................. 1228–1241

Effects of Natural and Sexual Selection on Adaptive Population Divergence and Premating Isolation in a DamselFly • Erin I. Stevenson, Fabrice Eroukhmanoff, and Magne Fægri .......................................................... 1242–1253

Multilocus Analyses of Admixture and Introgresion among Hybridizing Heliconius Butterflies • Marcus R. Koenig, Laura G. Young, Lauren M. Blume, and Lawrence E. Gilbert ................................................................. 1254–1268

Evolution of Intrinsic Growth Rate: Metabolic Costs Drive Trade-Offs between Growth and Swimming Performance in Meriditha menidae • Stephen A. Ainsworth, Sujinsi Chia, and David G. Coates ..................................................... 1269–1278

Genetic Population Structure and Call Variation in a Passerine Bird, the Satia Bowerbird, Philemon hypoxanthus violaceus • J. A. Nicholson, J. J. Austin, C. Moritz, and A. W. Godinzen ........................................... 1279–1290

Evolutionary Persistence of Phenotypic Integration: Influence of Developmental and Functional Relationships on Complex Trait Evolution • Rebecca L. Young and Alexander V. Badyaev ............................................... 1291–1299

BRIEF COMMUNICATIONS

Genetic Variation for Outcrossing among Caenorhabditis elegans Isolates • Henrique Tedone, Diego Manoil, and Patrick C. Phillips ................................................................. 1300–1305

Invertebrate Predation Selects for the Loss of a Morphological Antipredator Trait • Dirk Johannes Mikołajewski, Frank Johannson, Bianca Workshop, and Robby Store ................................................................. 1306–1310

Comparative Phylogenetic Analysis of Male Alternative Reproductive Tactic in Ray-Finned Fishes • Judith E. Marks and John C. Avise ..................................................... 1311–1316

ANNOUNCEMENTS ................................................................. 1317–1318

INSTRUCTIONS TO AUTHORS .................................................. 1320

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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.
INTRODUCTION

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 integration, 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 the current environment has a 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-megapixal digital camera (Camedia E-20 Olympus, Tokyo) mounted in a standard position, photographed under 103 magnification using a Leica (Bannockburn, IL) DC 300, or photographed under 7.53 magnification using an Olympic 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 correlations of FA in the mandible. FA was calculated for all $x$- and $y$-coordinates as the left minus the right side. 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 in 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 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-individual, and among-species variation), we evaluated consistency of landmark displacements within and outside of functional units by calculating 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:

$$\theta = \arccos \left( \frac{A \cdot B}{|A| \cdot |B|} \right)$$

where $A$ and $B$ are vectors containing the PC eigenvectors of each shape coordinate, and $|A|$ and $|B|$ are the length of $A$ and $B$; 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 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, 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: $t = 26.92, P = 0.01$; PC2: $t = 26.35, P = 0.01$; PC3: $t = 22.42, P = 0.05$). Likewise, comparisons of landmark displacements due to variation within and among individuals tended to be more similar within 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 integration 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 developmental 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.

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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.

REFERENCES


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.

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* 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°.
Figure 1. Phylogeny of nine species of *Sorex* shrews used in this study (based on Fumagalli et al. 1999).
Figure 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 ten for better visibility. Ellipsoids indicate functionally-integrated landmarks (i.e., muscle or connective tissue attachment sites).
Vector angle (arcsine °)

A)

B)

C)

FA vs. Individual Variation  Individual vs. Species Variation
Figure 3. Arcsine transformed vector angles (mean ± bootstrapped SD) of the landmark displacements for within-individual (FA) variation and among-individual variation in functionally integrated landmarks vs. non-functionally integrated landmarks and among-individual and among-species variation in functionally integrated landmarks vs. non-functionally 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 ($P < 0.05$).
Phylogenetic Distance (# of nodes) Between Species Pair

Developmental Integration

Functional Integration

Vector Angle (arcsine angle in °)

Phylogenetic Distance (# of nodes) Between Species Pair
Figure 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.
APPENDIX C: FUNCTIONAL EQUIVALENCE OF MORPHOLOGIES ENABLES
MORPHOLOGICAL AND ECOLOGICAL DIVERSITY

Published: *Evolution* (2007) 61: 2480-2492
Maternal care and human fitness

Grandparent helpers in Seychelles warbler

Sexual selection and reinforcement
Volume 61, Number 11, November 2007

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John J. Dennehy, Stephen T. Abedon, and Paul E. Turner

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Douglas W. Schelske and Paullette Bierzynke

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Marjorie D. Matocq and Peter J. Murphy

Geographic Variation in Avian Incubation Periods and Parental Influences on Embryonic Temperature
Thomas E. Martin, Sonya K. Auer, Ronald D. Bassar, Alina M. Niklison, and Penn Lloyd

Independent Evolution of Complex Life History Adaptations in Two Families of Fishes, Live-Bearing Halfbeaks (Zanichkovideridae, Beloniformes) and Poeciliidae (Cyprinodontiformes)
David Reznick, Robert Meredith, and Bruce B. Collette

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ABSTRACT

Diversity in organismal forms among taxa is thought to reflect distinct selection pressures across environments. The central assumption underlying this expectation is that taxa experiencing similar selection have similar response to that selection. However, because selection acts on trait function, taxa similarity in selection response depends crucially on the relationship between function and morphology. Further, when a trait consists of multiple parts, changes in function in response to selection can result from modification of different parts, and adaptation to the same environment might result in functional but not morphological similarity. Here, we address the extent to which functional and morphological diversity in masticatory apparatus of soricid shrews reflects a shared ecological characteristic of their diet type. We examine the factors limiting morphological variation across shrew species by assessing the relative contribution of trait function (biomechanics of the jaw), ecology, and phylogeny to species similarity in mandibular traits. We found that species that shared diet type were functionally but not morphologically similar. The presence of multiple semi-independently varying traits enabled functional equivalence of composite foraging morphologies and resulted in variable response to selection exerted by similar diet. We show that functional equivalence of multiple morphologies enabled persistence of differences in habitat use (e.g., habitat moisture and coverage) among species that specialize on the same diet. We discuss the importance of developmental and functional integration among traits for evolutionary diversification of morphological structures that generate equivalent functions.
INTRODUCTION

A central goal of evolutionary biology is to understand the origin of morphological diversity. Diversity in form is often associated with diversity of selection pressures experienced by taxa occupying different environments (Gatz 1979; Arnold 1983; Ricklefs and Miles 1994; Schluter 1996; Losos et al. 1998), such that morphological convergence among taxa is expected to result from similarity in selection pressures. However, convergence requires not only similarity in selection pressures, but also similarity in response to this selection (Gould 1985; Price et al. 2000; Van Buskirk 2002). Because selection acts at the level of trait function, trait similarity in response to selection depends on the concordance of functional and morphological variation (Koehl 1996; Schaefer and Lauder 1996; Alfaro et al. 2005).

When a function favored by selection in one environment can be achieved by only one phenotype, adaptation to the same environment across taxa should result in morphological convergence (e.g., Schluter and McPhail 1993; Koehl 1996; Losos et al. 1998). However, when functional equivalence is produced by distinct morphologies (e.g., when different morphologies can generate the same physiological output), ecological and functional similarity of taxa might be achieved by different responses among taxa to similar selection pressures (Schaefer and Lauder 1996; Wainwright et al. 2005), facilitating the evolution of morphological diversity (Alfaro et al. 2004). In addition, variation in response to selection enabled by the functional equivalence of morphologies may resolve trade-offs produced by a trait’s involvement in multiple functions and thus allow species to adapt to multiple aspects of their environment (e.g., Toro et al. 2004;
Alfaro et al. 2005; Westneat et al. 2005). Thus, species specializing to one ecological factor (e.g., particular diet) might be highly divergent in relation to other ecological factors (e.g., habitat type), and such differences may in turn generate diversity in responses to similar selection pressures (Schluter 2000).

Recent studies suggest that functional equivalence is an emergent property of complex morphologies (Alfaro et al. 2005; Wainwright et al. 2005). Because complex traits consist of multiple components that produce a particular function, change in that function can result from modification of any of the components of the complex trait resulting in a diversity of morphological solutions to the same functional requirement. Whereas, the importance of functional equivalence of morphologies for the evolution of diversity is well supported (Vermeij 1973; Koehl 1996; Wainwright et al. 2005), the factors that produce and limit this diversity are not well understood.

Here we test the hypotheses that functional equivalence facilitates morphological divergence among taxa that share an environmental characteristic, and allows otherwise ecologically divergent taxa to specialize on the same environmental resource. We predict that when functional similarity among taxa is achieved by multiple morphological solutions, species specializing on the same resource should be more similar in trait performance (e.g., biting force) than in trait morphology (e.g., skeletal structure). In addition, because functional equivalence of multiple morphologies enables variation in morphological response to shared selection pressures (e.g., Toro et al. 2004; Alfaro et al. 2005; Westneat et al. 2005), we predict that species experiencing similar functional requirements should likewise be more similar in trait performance than in characteristics
of habitat use. Moreover, variation in morphological response to shared selection
pressures should allow for the evolution of the same ecological specialization in distantly
related taxa. Finally, we address the limitations on the diversity of morphological
adaptations by examining the level of diversity across individual components of a
complex morphological trait, and discuss factors that may constrain the development and
evolution of some morphological variants.

We examine the association between morphology, ecology, and function in
mandibles of 15 species of soricid shrews (Fig. 1), insectivorous predators in which the
combination of rapid metabolism and variation across taxa in diet specialization results in
strong selection on the masticatory apparatus (Genoud 1988; Churchfield 1990; Zakharov
et al. 1991; Carraway and Verts 1994). Moreover, because diet specialization is
distributed across the phylogeny (Fig. 1), this system provides an opportunity to examine
ecological and evolutionary factors that influence interspecific diversity in both
morphology and function. Here, we first assess morphological similarity among species
with similar diet type. Second, we model mechanical potential of the jaw and associated
bite force to characterize the relationship between mandible morphology and function
(Carraway and Verts 1994). We examine concordance of morphological and functional
variation and establish functional equivalence of multiple morphologies. Lastly, to assess
constraints on across-species morphological variation associated with diet type, we
compare observed diversity in each mandible component across species that share diet
type with potential diversity generated using mandible morphologies simulated for each
species assuming no developmental or evolutionary constraints on proportionality among
components of the mandible.

MATERIALS AND METHODS

DATA COLLECTION

Morphological Measurements—In 15 species of Sorex shrews (species and sample sizes
shown in Fig. 1 and Table 1), we measured mandibles of fully grown individuals. Left
mandibles were placed on a slide and photographed at high resolution using an Olympus
(Tokyo, Japan), Camedia E-20, 5-megapixel digital camera mounted in a standard
position, or photographed under 10 x magnifications using a Leica DC 300
(Bannockburn, IL, USA) microscope (right mandibles were used in 24 individuals due to
damage on the left mandible). We scaled all images to standard size using a ruler
photographed with each mandible. Analyses of all images were conducted using Sgma-
Scan 5.0 Pro software (SPSS, Inc., Chicago, IL). From mandible images, we obtained
three measurements associated with bite force in shrews: coronoid–condyle length,
distance from condyle to molar bite point, and gape angle (Fig. 2; Carraway and Verts
1994; Carraway et al. 1996). In addition to their importance for overall mandible
function, these morphological measurements correspond with hypothesized
developmental and functional units and, are thus, semi-independent traits of the
mammalian mandible (Atchley and Hall 1991; Atchley 1993). We calculated mandible
size as centroid size using 15 landmarks commonly used in studies of shrew mandible
morphology (Fig. 2; also see Badyaev and Foresman 2000 for more details). Centroid
size was calculated as the square root of the summed squared distance between each landmark and the mandible center. We calculated repeatability of all measurements from the intraclass correlation coefficient (Lessels and Boag 1987) of ANOVA from a subset of 30 individuals (two from each species) measured three times. Repeatability of all measurements was > 97%.

Mechanical Potential Modeling—To model mechanical potential, we assumed the shrew mandible to be a simple lever in which the point of articulation between the mandible and skull (the condyloid process) serves as the fulcrum (after Fearnhead et al. 1955), and the distance from the tip of the coronoid process (the primary cite of insertion of *M. temporalis*; Badyaev et al. 2005; R. L. Young, unpubl. data) to the lower condyloid process represents the length of the muscle moment arm a. The muscle moment arm was set at an acute angle c to the resistance arm. The resistance moment arm was measured as the distance from the condyloid process to the tip of the bite point or the highest cusp on the first molar b (Fig. 2; after Carraway and Verts 1994; Carraway et al. 1996). This bite point is the major location of prey crushing and has been found to yield the highest values in empirical measurements of bite force in *Sorex* (R.L. Young, unpubl. data). From these measurements, mechanical potential, MP, was calculated as:

\[
MP = \frac{a}{b} \times \cos(\theta)
\]

where, \(\theta = 90 - c\), referred to as the force angle, and \(\cos \theta\) is the proportion of force directed at a right angle to the muscle moment arm (after Carraway and Verts 1994 and...
consistent with the general orientation of the *M. temporalis* originating at the suture point of the left and right parietal bones). This measure of function indicates that bite force is independent of (1) overall mandible size such that larger mechanical potentials signify greater bite forces and (2) any compensatory effects of musculature. Therefore, MP provides a measurement of potential function of the mandible and assumes (1) a single source of force responsible for jaw closure (i.e., the temporalis muscle on the coronoid process); (2) a constant input force across all samples; and (3) the application of force at a right angle to the muscle moment arm.

*Diet, Foraging, and Habitat Categorizations*—To categorize species diet type, habitat coverage and moisture association, and foraging type; we collected published data on diet type, stomach contents, species ranges, foraging behavior, and habitat associations (Table 2). Foraging type was defined as the location of foraging activity (Table 2). Habitat moisture levels were defined as either moist or dry and moist. Species strictly associated with swampy, boggy, or riverine habitats were labeled as moist. Because all species found in dry habitats (e.g., grass or scrublands) were also found in forest and riverine habitats associated with higher moisture levels, these species were categorized as dry and moist. Diet type was determined by prey hardness. Hard prey items (e.g., beetles and snails) were given a value of 1, soft prey items (e.g., earthworms, slugs, and larvae) a value of 0, and intermediate prey items (e.g., spiders and moths) a value of 0.5. Species with a mean diet score over 0.7 were categorized as “hard-bodied specialists,” species
with diet scores between 0.3 and 0.7 were categorized as “generalists,” and species with diet scores less than 0.3 as “soft-bodied specialists” (Table 2).

**Data Analysis**

*Phylogenetic analysis*— We constructed phylogenetic trees for the *Sorex* species used in the study using partial cytochrome *b* nucleotide sequences. We obtained 30 samples of 1011bp sequence from Genbank (two individuals from each the 15 *Sorex* species) and aligned them with the program Se-Al (Rambaut 1996). The water shrew, *Neomys anomalus*, was used as an outgroup. Phylogenetic reconstructions were performed using distance (neighbor-joining), maximum likelihood, and Bayesian analyses. These methods add to previous phylogenetic analyses of *Sorex* shrews by allowing calculation of branch lengths (Fumagalli et al. 1999; Ohdachi et al. 2006). Distance and maximum-likelihood analyses were determined using Paup version 4.0b10 (Swofford 2000). We first built neighbor-joining trees using two individuals from each species and the Tamura-Nei model of substitution. Once monophyly for each species was confirmed, one individual per species was used for maximum likelihood analyses. Maximum likelihood trees were reconstructed using the GTR+Γ+I model of nucleotide substitution, as determined by Modeltest (Posada and Crandall 1998) to be the best-fit model, and 1000 bootstrap replicates were run to assess phylogenetic support. We ran Bayesian analyses with Markov chain Monte Carlo sampling (MCMC) in MrBayes v3.0b4 (Huelsenbeck and Ronquist 2001) using the GTR+Γ+I model. The search was run with four chains for 1,000,000 generations sampling trees every 1000 generations, with the first 10,000
generations discarded. We ran the analysis three times, and the trees from all three runs were combined to determine posterior probabilities.

*Morphological and functional variation*— To examine the concordance of mandible morphology and diet type, we first used canonical discriminant analysis to summarize morphological variation between diet types, with each species weighted equally (SAS Institute Inc. 2004). Second, we calculated means for each of the four morphological measurements for each species (Table 1) and for each diet categorization. Means for each diet categorization were calculated as the mean of all species included in a diet category and group means of each measurement were compared using $t$-tests. All size and linear measurements were log transformed and angular measurements were arcsine transformed prior to analyses to achieve normal distribution (Sokal and Rohlf 1995). To examine concordance of function and diet type, we compared group mean mechanical potentials with $t$-tests.

*Consequences of morphological variability*— To examine the contribution of coronoid–condyle length, distance to molar, and force angle to overall morphological diversity, we compared mechanical potential and morphological variation for each trait among species using regression analysis. We examined the relationship between species’ diet categorization and their foraging and habitat type while controlling for phylogenetic distance between species (Table 2) by creating between-species dissimilarity matrices for foraging type, habitat coverage, and habitat moisture. Differences in diet type were
ranked as 0, 0.5, or 1, with 0 representing a species pair that share diet type, 0.5 representing a comparison between a generalist and a specialist, and 1 representing a comparison between hard and soft specialists. Phylogenetic distance was estimated using branch lengths from the phylogenetic tree (see Methods above). We calculated the distances between pairs of species by adding the lengths of the branches between them. This was done for distance, maximum likelihood, and Bayesian analyses. Between-species divergence in each of the remaining ecological characters, coverage, moisture and foraging type, were similarly calculated as a value between 0 and 1, 0 sharing the foraging or habitat type and 1 differing in foraging or habitat type.

Between-species dissimilarity matrices of morphological distance, mechanical potential, diet type, phylogenetic relatedness, and ecological divergence were compared using Mantel’s and partial Mantel’s tests. Partial Mantel’s test allows for the comparison of two matrices while controlling for the effect of the third (Smouse et al. 1986; Legendre and Legendre 1998); thus, it was used to control for species relatedness in comparisons between morphological, ecological, and functional distances. Morphological distances between all species pairs were estimated with Mahalanobis distances using canonical discriminant analysis. Functional difference between species pairs was the absolute value of the difference in mechanical potential. Ecological divergence between species was measured as dissimilarity in foraging, coverage, and moisture type combined. Each species pair was scored with a value between 0 and 1, species pairs that share all habitat (i.e., coverage and moisture) and foraging characteristics were scored as 0, and species pairs with divergent habitat and foraging characteristics were scored as 1. Ecological
dissimilarity scores for species pairs sharing some habitat or foraging characteristics were calculated as the average of the species-pair dissimilarity scores for coverage, moisture, and foraging characteristics. All matrix correlations (Mantel’s statistic) were estimated with R (R Development Core Team 2004; Oksanen et al. 2005).

*Constraints on diversity mandible components*—To characterize constraints on morphological variation in relation to diet, we compared observed diversity in morphological adaptation to null models of predicted potential morphological diversity for each diet type. To estimate potential diversity for each diet type, 20 morphologies were randomly generated for each species such that their coronoid–condyle length (a), distance to bite point (b), and force angle (q) were limited within the range of values observed for the species and their mechanical potential fell within the interquartile range for the species. This was repeated 1000 times for each species. First, for each iteration, we calculated means for all three morphological variables. Second, one iteration was sampled without replacement for each species within a diet category, and the interspecific means, coefficients of variation (CVs), and variances were calculated for each mandible character until all iterations had been selected. Finally, observed means, CVs, and variances for each trait and diet type were compared to simulated distribution of means, CVs, and variances using z-tests. Among species sharing diet type, variation in each of the three morphological character was considered constrained when observed CV and variance for a component of the mandible were lower than those generated by the model.
RESULTS

Phylogenetic Analysis

Distance (neighbor-joining), maximum likelihood, and Bayesian methods all yielded the same topology with good phylogenetic support for most clades (Fig. 1), corroborating phylogenetic relationships among soricid shrews found in a previous analysis (Fumagalli et al. 1999; Ohdachi et al. 2006).

Morphological and Functional Variation

Morphological divergence between diet types differed among the four measured traits, coronoid–condyle length, distance to the first molar, force angle, and mandible size (Tables 1 and 2; Figs. 3, 4). The first canonical axis accounted for 94.4% of the differences between diet types and consisted of variation in force angle, $\theta$, and coronoid–condyle length, $a$, whereas the second canonical axis contributed only 2.9% of the morphological divergence between diet types and consisted of distance to the first molar, $b$ (Figs. 2, 3). Force angle differed among all three diet categories (general vs. hard: $t = 5.5, P < 0.001$; generalist vs. soft: $t = -7.1, P < 0.001$; hard vs. soft: $t = -9.8, P < 0.001$) and distance to the bite point did not differ between groups (general vs. hard: $t = -0.27, P = 0.8$; generalist vs. soft: $t = -0.89, P = 0.4$; hard vs. soft: $t = -0.08, P = 0.9$; Fig. 4). Hard specialists differed in coronoid–condyle length (Fig. 4; general vs. hard: $t = -3.1, P = 0.003$; generalist vs. soft: $t = -0.58, P = 0.6$; hard vs. soft: $t = 2.91, P = 0.005$), and mandible size differed between generalists and soft specialists (Fig. 4; general vs. hard: $t = -0.9, P = 0.4$; generalist vs. soft: $t = -3.5, P < 0.001$; hard vs. soft: $t = -0.9, P = 0.4$).
Although coronoid–condyle length, distance to incisor, and mandible size did not differ among all diet categorizations (Fig. 4), mechanical potential differed among all three diet categories (general vs. hard: \( t = -2.13, P = 0.06 \); generalist vs. soft: \( t = 3.63, P < 0.05 \); hard vs. soft: \( t = 4.49, P = 0.05 \); Fig. 5).

**Functional Equivalence of Multiple Morphologies**

Across study species, different mandible morphologies produced the same mechanical potential. As a result, functional, but not morphological divergence was correlated with species differences in diet type (Table 4, comparisons of mechanical potential and morphology indicated that the relationship differed among traits). Across taxa, variation in morphological response to diet type was produced by modification of some traits, but persistence of others (Fig. 6). Both coronoid–condyle length \( (r^2 = 0.39, P = 0.02) \) and force angle \( (r^2 = 0.67, P < 0.001) \) positively correlated with mechanical potential, whereas distance to the bite point \( (r^2 = 0.13, P = 0.18) \) and mandible size \( (r^2 = 0.11, P = 0.24) \) did not vary with mechanical potential (Fig. 6). There was no correlation among matrices of diet type, foraging, and habitat characteristics indicating no association among ecological characteristics across species (Table 3). Phylogenetic divergence among species was not concordant with species divergence in morphology, function, diet type, and ecology \( (r = -0.18, P = 0.85; r = -0.23, P = 0.98; r = -0.14, P = 0.14; r = -0.12, P = 0.89) \). Morphological divergence was not consistent with ecological divergence among species; matrix correlations between Mahalanobis distances of species pairs did not vary with ecological divergence among species (i.e., morphological distance
was not consistent with divergence in diet type or habitat coverage, moisture, and foraging characteristics among taxa; Table 4).

**Constraints on Diversity in Mandible Components**

Comparisons of observed and potential variation in each trait show that response to shared diet varied across traits for some diet types (Fig. 7). For coronoid–condyle length, observed variation was similar to the potential variation in generalists (CV: $z = -0.45, P = 0.33$; variance: $z = -0.56, P = 0.29$) and hard specialists (CV: $z = -1.45, P = 0.07$; variance: $z = -1.13, P = 0.13$). However, in soft specialists, variation of coronoid-condyloid values among species was greater than expected (CV: $z = 8.31, P > 0.99$; variance: $z = 14.83, P > 0.99$). Observed variation in distance to the molar bite point was greater than the potential variation in both generalists and soft specialists (generalists CV: $z = 1.87, P = 0.97$, variance: $z = 1.89, P = 0.97$; soft specialists CV: $z = 7.8, P = 0.99$, variance: $z = 8.3, P = 0.99$), and indistinguishable from potential variation in hard specialist (CV: $z = 0.23, P = 0.59$, variance: $z = 0.34, P = 0.63$). For force angle, the observed variation was consistent with potential variation in generalists and hard specialists (generalists CV: $z = 0.15, P = 0.56$, variance: $z = 0.1, P = 0.54$; hard specialists CV: $z = -0.95, P = 0.17$, variance: $z = -0.89, P = 0.19$), and was greater than expected for soft specialists (CV: $z = 7.59, P = 0.99$, variance: $z = 17.55, P > 0.99$). CVs and variances yielded similar results, thus only variances are shown (Fig. 7).

**DISCUSSION**
Discordance in patterns of ecological and morphological diversity across taxa can result from variation in response to similar selection pressures. Such variation is often attributed to historical contingency—the constraining effects of taxa-specific evolutionary histories (Schluter and Nagel 1995; Losos et al. 1998; Langerhans and DeWitt 2004). However, if equivalent adaptive solutions are accomplished by multiple morphologies, then morphological variation among taxa specializing on the same environment can result from the ability of a trait to adapt to different combinations of environmental characteristics across taxa, such that, diversity in form would result from trait versatility in adaptation rather than constraints imposed by evolutionary history. In this case, morphological diversity depends on trait lability and the degree of independence among the components of a trait (Vermeij 1973). Thus, characterizing the relationship between morphological and functional variation can not only distinguish among alternative explanations for the evolution of morphological diversity (e.g., historical contingency vs. versatility), but also provide insight into the limitations on diversity of a morphological structure (Vermeij 1973; Wagner 2001; Wainwright et al. 2004; Alfaro et al. 2005; Westneat et al. 2005; Young and Badyaev 2006).

We tested the a priori prediction that the combination of functional equivalence of form, shared functional requirements, and ecological diversity among shrew species should result in different evolutionary responses among taxa to similar selective pressures, ultimately producing functional but not morphological similarity among species that share a diet type. Our results supported this prediction—species with distinct diets differed in mandible function (Fig. 5), but were similar in mandible morphology.
Similarly, we found that within diet type, multiple individual morphologies generated identical mechanical potentials supporting the hypothesis that functional equivalence of multiple morphologies might be common in complex structures (Alfaro et al. 2005; Wainwright et al. 2005). As predicted, variation in response to selection facilitated different morphological solutions to selection exerted by a diet type (Table 4) and allowed for the evolution of diet specialization across distantly related species (Tables 2; Fig. 1). Moreover, functional equivalence of morphologies may have facilitated diet specialization among taxa with otherwise dissimilar ecological characteristics, because between-species similarity in diet type differed from between-species similarities in moisture, coverage, or foraging (Table 3). We found that functional equivalence of morphologies resulted from high variability in mandible characters among species that share diet type, because for each diet type variance in at least one morphological character was greater than expected. Interestingly, across diet type this high variability was found in different mandible components. Together, these results indicate that variation in morphological adaptation requires lability in only a subset of trait components (Fig. 7).

When a complex morphological structure consists of multiple components, such complexity can facilitate the evolution of differing morphological responses to shared selection pressures, and the variability in selection response should depend on the number of semi-independently varying parts (Vermeij 1973) and the strength of their integration (Vermeij 1973; Lande and Arnold 1983; Bonner 1988; Raff and Raff 2000). Theory predicts that the correlational structure among components of a complex trait should be
consistent with functional relationships among components (Cheverud 1982, 1988, 1996; Wagner 1996), and thus the generation of variation during development and over evolutionary time in complex morphologies should reflect functional relationships among components. Here, we found that mandible traits of shrews differed in their response to changing functional requirements such that some characters were more variable across diet types (e.g., force angle, Fig. 4) than others. Variation in lability among mandible characters across diet types may reflect difference among components in their contribution to overall mandible function (Atchley and Hall 1991). These distinct roles may favor the evolution of weak developmental and genetic integration among these structures through exploitation of variation across mandible components generated as a result of variation in functional roles during trait development. For example, during development of skeletal traits, epigenetic interactions between muscle and bone strongly influence morphological structure (e.g., see Herring 1993; Huiskes 2000), and regions of the same skeletal structure often experience distinct extrinsic pressures from differences in growth of surrounding tissues (e.g., vascular development or brain growth), or attachment and loading of connective tissues (i.e., tendons and ligaments; Henderson and Carter 2002). Furthermore, temporal and spatial distribution of mechanical stresses (e.g., muscle loading) may enable independence in development among components of the mandible through differential growth (Henderson and Carter 2002; Badyaev et al. 2005; Zelditch 2005) and decoupling of ossification timing among units (Smith 2002). This variation in stresses can result in differences in gene expression regulating rates of cell division and differentiation among components of a skeletal trait, and strongly influence
correlation structure among regions of the mandible (reviewed in Henderson and Carter 2002; Zelditch 2005; Young and Badyaev 2007). Here, we found that later ossifying tissues (e.g., the coronoid and condyloid processes) were the most variable across diet types (Fig. 4, force angle and coronoid–condyloid length; Atchley 1993; Ramaesh and Bard 2003), suggesting that timing of ossification may be important in determining correlation structure among mandible components. Furthermore, variation in trait response to changes in functional requirements may reflect differences in sensitivity of trait development to epigenetic signals. We found that force angle and coronoid–condyloid length, traits associated with the muscle attachment region of the mandible, were the most variable both within and among diet categories (Fig. 7). These results corroborate previous findings of the importance of epigenetic signals and suggest that interactions between muscle and bone are crucial in determining developmental relationships among components of the shrew mandible (Badyaev et al. 2005; Young and Badyaev 2006, 2007).

Given our finding of variation in morphological response to selection for diet type, we expected to find high variability in mandible components across species that share diet type. In fact, we found few constraints on diversity of morphological solutions to diet specialization across taxa—observed variation was indistinguishable from or higher than potential variation simulated with the null model of diversity (e.g., force angle, Fig. 7). Interestingly, however, means of some traits differed from those predicted under the simulation model (e.g., mean force angle, Fig. 7) suggesting bias in the production of variation in this system. Consistent with the idea that diversity in form
among taxa specializing on the same diet can result from versatility of a trait to adapt to
different combinations of environmental characteristics across taxa, such biased
production of some morphologies is expected when some traits are used in multiple
functions and are favored by selection for other jaw functions (e.g., grooming or social
interactions). Overall, our findings of high realized levels of morphological diversity
among taxa experiencing similar selection suggest high lability in morphological
response to selection in this system.

Variation in adaptive response to changing functional requirements can generate
morphological diversity among taxa by allowing functional similarity of multiple
morphologies and, correspondingly, unique evolutionary solutions to similar selective
pressures. The findings that mandible characters exhibiting the greatest variability are
also the most sensitive to external effects (e.g., epigenetic interaction between muscle and
bone, Atchley 1993; Ramaesh and Bard 2003), suggest that differences among traits in
response to changing functional requirements may result from variation in timing and
environmental sensitivity of trait development.

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Table 1. Species sample size and means (coefficient of variation) for morphological and functional traits measured in this study.

<table>
<thead>
<tr>
<th>Species</th>
<th>N</th>
<th>Coronoid-condyle length [mm]</th>
<th>Distance to molar [mm]</th>
<th>Force angle [°]</th>
<th>Mandible size [centroid]</th>
<th>Mechanical potential</th>
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<td>5.20(7.9)</td>
<td>30.9(9.0)</td>
<td>10.29(5.2)</td>
<td>0.488(8.9)</td>
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<tr>
<td><em>S. hoyi</em></td>
<td>20</td>
<td>2.77(4.4)</td>
<td>4.56(4.7)</td>
<td>31.7(5.9)</td>
<td>9.03(5.2)</td>
<td>0.517(6.4)</td>
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<td><em>S. minutus</em></td>
<td>20</td>
<td>2.83(4.8)</td>
<td>5.06(6.5)</td>
<td>31.4(10.2)</td>
<td>10.42(4.3)</td>
<td>0.477(6.9)</td>
</tr>
<tr>
<td><em>S. monticolus</em></td>
<td>15</td>
<td>3.08(13.9)</td>
<td>5.36(8.6)</td>
<td>38.8(3.6)</td>
<td>11.11(10.9)</td>
<td>0.446(7.0)</td>
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<tr>
<td><em>S. pacificus</em></td>
<td>20</td>
<td>5.02(3.9)</td>
<td>7.35(3.6)</td>
<td>26.7(11.5)</td>
<td>15.13(3.6)</td>
<td>0.610(4.1)</td>
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<td><em>S. palustris</em></td>
<td>20</td>
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<td>6.85(2.9)</td>
<td>29.9(12.4)</td>
<td>14.38(4.8)</td>
<td>0.522(5.4)</td>
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<td><em>S. trowbridgii</em></td>
<td>18</td>
<td>3.45(5.0)</td>
<td>6.00(4.2)</td>
<td>31.2(5.4)</td>
<td>12.16(4.0)</td>
<td>0.491(5.2)</td>
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<td><em>S. tundrensis</em></td>
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<td>3.46(3.1)</td>
<td>6.04(3.2)</td>
<td>34.7(7.4)</td>
<td>12.4(3.2)</td>
<td>0.470(3.5)</td>
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<td>14</td>
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<td>5.47(4.1)</td>
<td>31.8(8.5)</td>
<td>10.9(6.5)</td>
<td>0.509(5.6)</td>
</tr>
<tr>
<td>Species</td>
<td>Diet type</td>
<td>Foraging type</td>
<td>Habitat moisture</td>
<td>Habitat coverage</td>
<td>References</td>
<td></td>
</tr>
<tr>
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<td>------------------------------------------------</td>
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<tr>
<td><em>S. alpinus</em></td>
<td>soft bodied specialist</td>
<td>surface and litter</td>
<td>moist habitats</td>
<td>open and closed habitats</td>
<td>(Hutterer 1982; Wilson and Reeder (eds) 1993)</td>
<td></td>
</tr>
<tr>
<td><em>S. arcticus</em></td>
<td>hard bodied specialist</td>
<td>surface and litter</td>
<td>moist habitats</td>
<td>closed habitats</td>
<td>(Jackson 1961; Peterson 1966)</td>
<td></td>
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<tr>
<td><em>S. caecutiens</em></td>
<td>generalist</td>
<td>surface</td>
<td>dry and moist habitats</td>
<td>open and closed habitats</td>
<td>(Churchfield 1990; Churchfield et al. 1999)</td>
<td></td>
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<td><em>S. cinereus</em></td>
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<td>surface and litter</td>
<td>moist habitats</td>
<td>closed habitats</td>
<td>(French 1984) (Pagels et al. 1994; Brannon 2000; Bellocq and Smith 2003)</td>
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<td><em>S. coronatus</em></td>
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<td>surface and litter</td>
<td>moist habitats</td>
<td>closed habitats</td>
<td>(Yalden et al. 1973; Churchfield 1990)</td>
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</tr>
<tr>
<td><em>S. fumeus</em></td>
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<td>litter and soil</td>
<td>moist habitats</td>
<td>closed habitats</td>
<td>(Whitaker and Cudmore 1987; Churchfield 1990; Brannon 2000)</td>
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<td><em>S. haydeni</em></td>
<td>generalist</td>
<td>surface and litter</td>
<td>moist habitats</td>
<td>open habitats</td>
<td>(van Zyll de Jong 1980; Clark and Stromberg 1987)</td>
<td></td>
</tr>
<tr>
<td><em>S. hoyi</em></td>
<td>hard bodied specialist</td>
<td>surface, litter, and soil</td>
<td>dry and moist habitats</td>
<td>closed habitats</td>
<td>(Clark and Stromberg 1987; Whitaker and Cudmore 1987; Churchfield 1990; Crowcroft 1955; Yalden 1981; Churchfield 1990, 1994)</td>
<td></td>
</tr>
<tr>
<td><em>S. minutus</em></td>
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<td>litter and soil</td>
<td>moist habitats</td>
<td>closed habitats</td>
<td>(Whitaker and Maser 1976; Terry 1981; Carraway and Verts 1994)</td>
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</tr>
<tr>
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<td>litter</td>
<td>moist habitats</td>
<td>closed habitats</td>
<td>(Whitaker and Maser 1976; Carraway and Verts 1994)</td>
<td></td>
</tr>
<tr>
<td><em>S. pacificus</em></td>
<td>hard bodied specialist</td>
<td>surface</td>
<td>dry and moist habitats</td>
<td>closed habitats</td>
<td>(van Zyll de Jong 1983; Beneski and Stinson 1987; Clark and Stromberg 1987)</td>
<td></td>
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<td>closed habitats</td>
<td>(Whitaker and Maser 1976; Carraway and Verts 1994)</td>
<td></td>
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<tr>
<td><em>S. trowbridgii</em></td>
<td>generalist</td>
<td>litter and soil</td>
<td>dry habitats</td>
<td>closed habitats</td>
<td>(Youngman 1975; van Zyll de Jong 1983)</td>
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<tr>
<td><em>S. tundrensis</em></td>
<td>soft bodied specialist</td>
<td>surface, litter, and soil</td>
<td>dry and moist habitats</td>
<td>closed habitats</td>
<td>(Terry 1981; Whitaker et al. 1983; Gillihan and Foresman 2004)</td>
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</tr>
<tr>
<td><em>S. vagrans</em></td>
<td>generalist</td>
<td>surface and litter</td>
<td>moist habitats</td>
<td>closed habitats</td>
<td>(Terry 1981; Whitaker et al. 1983; Gillihan and Foresman 2004)</td>
<td></td>
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</table>
Table 3. Comparison of between-species divergence in diet type, foraging type, habitat moisture and coverage characteristics to test for concordance in use of resources and habitat type among taxa. Mantel’s Statistic ($r$) below the diagonal, $P$-values are shown above diagonal.

<table>
<thead>
<tr>
<th></th>
<th>Diet type</th>
<th>Foraging type</th>
<th>Habitat moisture</th>
<th>Habitat coverage</th>
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<td>0.8</td>
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<tr>
<td>Foraging type</td>
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<td>.</td>
<td>0.2</td>
<td>0.8</td>
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<tr>
<td>Habitat moisture</td>
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<td>0.07</td>
<td>.</td>
<td>0.8</td>
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<tr>
<td>Habitat coverage</td>
<td>-0.09</td>
<td>-0.15</td>
<td>-0.07</td>
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</tbody>
</table>
Table 4. Comparison of morphological and functional (MP, mechanical potential) divergence among species controlling for species relatedness. We examine the relationship among trait morphology and function and organismal diet and environmental characteristics to identify factors that important for species divergence in morphology and function. Partial Mantel’s Statistic ($r$) below the diagonal, $P$-values above diagonal. Significant correlations are highlighted in bold.

<table>
<thead>
<tr>
<th></th>
<th>Mahalanobis distance</th>
<th>Difference in MP</th>
<th>Diet type</th>
<th>Habitat type</th>
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<td><strong>0.001</strong></td>
<td>0.3</td>
<td>0.59</td>
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<td>Difference in MP</td>
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<td><strong>0.006</strong></td>
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<td><strong>0.36</strong></td>
<td>.</td>
<td>0.9</td>
</tr>
<tr>
<td>Habitat type</td>
<td>-0.04</td>
<td>-0.12</td>
<td>-0.14</td>
<td>.</td>
</tr>
</tbody>
</table>
Figure 1. The maximum likelihood phylogeny of the Sorex species used in this study. The numbers at each node in the phylogeny represent Bayesian posterior probabilities/maximum likelihood bootstrap values (if >50%).
Figure 2. Morphological measurements used to calculate bite force and characterize morphology: a: coronoid-condyle length, b: distance to the highest cusp on the first molar, c: gape angle, and θ: force angle. Mechanical potential MP = a/b * cos θ.
Figure 3. Canonical discriminant analysis of morphological variation among diet types. Abscissa is the first canonical axis; ordinate is the second canonical axis. Shown are mean ± 1 standard deviations for each diet category for the first two canonical axes. Closed circles show diet generalists, grey circles – hard specialists, and open circles – soft specialists.
Figure 4. Morphological divergence among diet types. Shown are mean ± 1 standard error. Closed bars indicate diet generalists, grey bars – hard specialists, and open bars – soft specialists. Lines with asterisks connect means that are significantly different (α < 0.05).
Figure 5. Comparison of mean mechanical potential among diet categories. Shown are mean ± 1 standard error. Closed bars indicate diet generalists, grey bars – hard specialists, and open bars – soft specialists. Lines with asterisks connect means that are significantly different (α < 0.05); blank lines indicate differences in means (α < 0.07).
Figure 6. Relationship between mandible morphology and mechanical potential.

Coronoid-condyle length and force angle are significantly correlated with mechanical potential, and mandible size and distance to the molar are not correlated with mechanical potential. Lines indicate significance ($P < 0.05$) in the regression of the morphological characters on mechanical potential.
Figure 7. Comparison of observed and potential trait means and variances for each trait and diet type. Top row: coronoid-condyle length means, second row: coronoid-condyle length variances, third row: distance to molar means, fourth row: distance to molar variances, fifth row: force angle means, and bottom row: force angle variances. Distributions show the frequency of means and variances generated by simulation. Observed means or variances and significance are given on each distribution.
APPENDIX D: DEVELOPMENTAL PLASTICITY LINKS LOCAL ADAPTATION
WITH DIVERSIFICATION IN FORAGING MORPHOLOGY OF SHREWS
ABSTRACT

Developmental plasticity is thought to reconcile the constraining role of natural selection in maintaining local adaptation with evolutionary diversifications 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 from sporadic early embryonic motility to directed functional effects at late embryonic stages. In species with short generation times, delayed skull maturation extends the directing effects of muscle activity on formation of foraging morphology into early adulthood, providing a unique opportunity to directly examine the links between plasticity of bone development, ecological adaptations, and evolutionary diversification in foraging morphology. Here we show that epigenetic regulation of bone growth and maturation in Soricid shrews enables both development of local adaptations and evolutionary diversification in mandibular morphology. We contrast the effects of muscle stimulation on early- and late-maturing elements of foraging apparatus to show that the shape of late maturing elements is more affected by adaptive functional requirements of bite force than is morphology of early ossifying traits. We further show that divergence in foraging morphology across shrew species occurs along the directions delineated by inductive effects of locally adaptive bite force on bone formation during late ontogeny within species. The results show how evolved developmental plasticity can link maintenance of precise local adaptations under natural selection with observed evolutionary diversification in development and morphology.
Differences in resource availability across environments or due to competition within an environment favor the evolution of morphological diversity (Simpson 1944; Brown and Wilson 1956; Losos 2000; Schluter 2000). The efficacy of divergent natural selection in maintaining local adaptations depends crucially on ontogenetic variation (Waddington 1941; West-Eberhard 2003). On one hand, greater developmental plasticity enables exploitation of diverse ecological resources fueling extensive adaptive radiations (West-Eberhard 1989; West-Eberhard 2003). On the other hand, selection for precise local adaptation hinders further developmental innovations enforcing stasis of adapted forms (Vermeij 1996). Developmental plasticity is thought to link local adaptation and evolutionary diversifications – thus providing continuity in evolutionary processes (West-Eberhard 2003) – but empirical documentations of such link and its mechanistic underpinnings are rare.

Epigenetic regulation of skeletal development by attached musculature provides an opportunity to study evolutionary consequences of developmental plasticity, because ontogenetic variation in internal and external causes of the muscle activity and their corresponding effect on skeletal formation link directly developmental plasticity and timing of natural selection for local adaptation. 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 1991; Enlow 2000). Late in ontogeny, when muscle actions are partially guided by functional demands, their effects on bone remodeling and skeletal morphology are more directly linked to locally
adaptive functions (Frost 1987). Thus, the role of muscle-bone interactions in development of local adaptations depends on the timing of bone formation in relation to onset of functional use, and evolutionary consequences of epigenetic inputs due to local functional requirements should be evident in adaptive radiations across taxa.

Particularly instructive in this respect are mammalian species in which delayed ossification of skull’s foraging apparatus extends the effects of muscle activity on formation of mandibular morphology into post-weaning independent foraging, enabling examination of directing role of ecological variation on bone development. In soricid shrews (Sorex sp.), mandible growth and development occurs late in ontogeny (Vogel 1973; Foresman 1994; Masuda and Yohro 1994) and coincides temporally with the independent foraging (Masuda and Yohro 1994). Here, we test the hypothesis that the evolved developmental plasticity, where the development of late maturing mandibular components is influenced by local functional demands of foraging, links morphological adaptation and diversification within and across species. We contrast the effects of foraging-linked muscle activity on development of early and late ossifying mandibular components and compare the contribution of the early and late ossifying mandibular components to evolutionary diversification in mandible morphology in shrews. We predict that variation in late ossifying regions of the mandible should enable both, development of locally adaptive mandibular morphology within a species and divergence in morphology across species.

Early and late maturing regions of the shrew mandible (Fig. 1) are both exposed to muscle activity and other epigenetic inputs associated with foraging (Figs. S1);
however, because the two mandible regions experience these inputs at distinct
developmental stages the consequences of muscle activity should differ. We compared
the effects of muscle activity on mandible size and shape variation – two traits of know
importance for bite force (Young et al. 2007, see also Fig. 2 C-F) – in the early and late
ossifying regions of the mandible in *Sorex monticolus*. Muscle activity associated with
biting (Fig. S2) had a large influence on development of mandibular form (i.e., size and
shape) in late, but not early ossifying regions of the mandible (Table 1 – size – early
ossifying: $F = 0.04, P = 0.99$, late ossifying: $F = 2.68, P = 0.05$; Fig. 2A – shape).
Distinct developmental effects of muscle activity in the early and late ossifying regions of
the mandible influenced expression of variation in the two regions. The early and late
maturing regions of the mandible showed equivalent levels of variation among
individuals (Fig. 2B), suggesting in similarities in developmental variability between the
two regions. At the same time, the late maturing region exhibited significantly lower
levels of fluctuating asymmetry (FA, random accumulation of developmental errors or
deviations from bilateral symmetry of the left and right sides of the mandible; Fig. 2B)
(Van Valen 1962). Reduced FA in the late maturing region (Fig. 2B, developmental
errors) suggests that early onset of foraging-linked muscle activity in the late maturing
region directs the development of variation minimizing the accumulation of random
developmental errors. This role of muscle activity in channeling the developmental
effects of environmental inputs (e.g., muscle action or environmental stress) has known
consequences for morphological evolution in this system (Badyaev and Foresman 2000;
Badyaev et al. 2005). Importantly, we found that differences between early and late
ossifying regions of the mandible in the morphological effects of muscle activity resulted from adjustments of bone growth (Fig. 2 A, B) and not post-ossification remodeling, as histological evidence revealed similar levels of bone remodeling – estimated as secondary osteon and osteocyte density – in the early and late maturing regions of the mandible (Fig. S3). These results show that muscle function 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 function.

In shrews, the functional demands on mandibular form vary widely across environments in relation to diet (Young et al. 2007). We found that ontogenetic timing of bone formation resulted in distinct developmental effects of muscle activity associated with the local demands of foraging in the early and late maturing regions of the mandible (Figs. 2 A, B). We examined the consequences of these effects by comparing the importance of mandible size and shape – two traits influenced by muscle activity (Table 1, Fig. 2A) – in the early and late maturing regions for generating bite force in a population of S. monticolus (Fig. 1C). Distinct morphological effects of muscle activity in the early and late ossifying regions of the mandible translated into differential contribution of the two regions to bite force. Size and shape of the late but not early ossifying region of the mandible strongly contributed to bite force (Fig. 2 C-F). This finding indicates that muscle action can adjust morphology of the late maturing region of the mandible to meet the functional requirements of the local environment. Moreover, these adjustments occur by muscle-induced modification of bone growth and development of the late maturing region rather than through post-ossification remodeling.
of the structure (Table 1; Figs. 2A, S3). These results show that heterochrony of bone
development between mandibular regions and the resulting muscle-induced
developmental plasticity of the late ossifying regions enable the production of locally
adaptive morphologies and these processes should be important for adaptive
diversification in shrews.

Early and late ossifying regions of mandible differ in their association with
foraging strategies and diets among shrew species (Young et al. 2007). To examine
whether muscle-bone developmental interactions in the late ossifying mandibular region
underlies this association and corresponding adaptive diversification across taxa, we
examined interspecific variation in size and shape and concordance of the direction of
species divergence with direction of muscle-induced variation within species in early and
late ossifying regions of the mandible 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. After controlling for phylogenetic relatedness of the taxa, we
found first that interspecific variation in size was greater in the late versus early ossifying
region of the mandible (Fig. 3A). Increased interspecific variation in size of the late
maturing region of the mandible likely reflects differences among taxa in diet, as within-
species size variation in the late ossifying region of the mandible strongly associated with
bite force (Fig. 2E). Size of the late ossifying region of the mandible is also strongly
influenced by the muscle activity associated with foraging (Table 1), linking muscle-
induced development of morphological variation with interspecific divergence in
mandible morphology. Second, despite similar levels interspecific variation in shape
between the two mandibular regions (Fig. 3B), the direction of interspecific variation in shape of the late maturing region of the mandible was highly concordant with the direction of muscle-induced variation within species (Fig. 3C). Within species, this muscle-induced variation in the late maturing region of the mandible resulted in individual variation in bite force (Fig. 2A, F), indicating that species divergence occurs along the lines delineated by epigenetic effects associated with the functional demands of foraging. Our results indicate that divergence among taxa in diet and associated muscle activity can direct the development of adaptive morphological diversification among taxa and confirm the importance of environment-induced variation in divergence among Sorex taxa (Badyaev and Foresman 2000; Young and Badyaev 2006).

Taken together, these results show how epigenetic regulation of bone formation can generate both adaptation and diversification in mandible morphology. We found that morphological diversification across species is concordant with intraspecific patterns of epigenetically-induced adaptation, development of the late ossifying region of the mandible was more affected by functionally important muscle activity, and the resulting mandible form closely covaried with bite force. Further, high developmental plasticity of the late ossifying region of the mandible might have enabled extensive diversification of mandibular morphology across shrew species experiencing contrasting functional and ecological requirements of foraging. The conserved role of muscle-bone interaction in skeletal development suggests that changes in plasticity of skeletal traits likely evolve through heterochronic shifts in the timing of bone development in relation to internal and external functional demands (Hall 1984; Meyer 1987; Young and Badyaev 2007).
FUNCTIONAL CHARACTERISTICS OF THE SHREW MANDIBLE

Mechanics and Musculature – Foraging in shrews consists of jaw movements associated with capture (jaw opening), initial crushing of prey, and prey processing (positioning, shearing, and grinding of prey) (Dötsch 1982; Dötsch 1994) (Fig. S1). Several muscles contribute to jaw movement (reviewed in Sharma 1958); however, over 90% masticatory function results from action of three muscles: Musculus temporalis, M. masseter, and M. digastricus (Fig. S1 A, B, and D, respectively) (Dötsch 1985; Dötsch 1994). We measured epigenetic inputs associated with foraging using these muscles and their associated mechanical influences on the mandible (described in Fig. S1).

DATA COLLECTION

Bite Force Performance – We captured 26 montane shrews (Sorex monticolus) in the Pinaleño and Jemez mountain ranges of Arizona and New Mexico in June-August 2005. Upon capture, we measured in vivo bite force using bite plates attached to Kistler force transducer (type 9203) and charge amplifier (type 5995, Kistler Inc., 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 1mm between all repeated measures and all individuals.
Muscle function—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. S1). 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. S1B). Next, muscles were dissected off of the mandible, weighed (with 0.01mg resolution) using a Mettler Toledo AB135-S/FACT balance (Columbus, OH), immersed in 40% nitric acid (HNO₃) until muscle fibers separated (24-30 hours), and stored in a 50% aqueous glycerol solution (after Herrel et al. 1998a). Muscle fibers were photographed under 10-12.5x magnifications using a Leica DC 300 (Bannockburn, IL), sizes were standardized using a ruler photographed along with the fibers. Fiber length of each muscle was the mean of measurements of twenty unique fibers. Force of each muscle was calculated as the product of the physiological cross section (cm²) and a force conversion factor (*C* = 25) (Herzog 1994), where physiological cross section was the quotient of muscle mass (g) and mean fiber length (cm) (after Herrel et al. 1998a). We estimated function exerted by each muscle on the mandible as the force distributed across the area of attachment. Area was measured as the ellipse formed by the length and height of the muscle attachment. We examined contribution of each of the three muscles to bite force performance by regressing bite force on muscle stress.
**Skull Morphology**— Left and right mandibles were separated, placed on a slide, and photographed under 10x 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. 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 based on knowledge of developmental sequence and timing in the mandible (Fig. 1, Vogel 1973; Yamada and Yohro 1988; Foresman 1994; Masuda and Yohro 1994). We estimated size of each mandibular region by calculating centroid size separately for the two regions as the square root of the summed squared distance of each landmark to the center of the region. Developmental variation in mandible shape was measured using covariation of fluctuating asymmetry (FA, or deviations from bilateral symmetry) between the left and right sides of the mandible for all 19 landmarks (see additional discussion in the text). Because trait variation due to FA results from random perturbations during development (reviewed in Hallgrímsson 1999), FA variation should be randomly distributed unless traits share direct developmental links (Klingenberg et al. 2001; Klingenberg 2003).

**Skull Histology** — To assess differences between the early and late ossifying regions of the mandible in bone remodeling, formalin-fixed mandibles were decalcified and sectioned sagitally (5µm, see Fig. 3A). Sections were stained with hematoxylin and eosin and photographed under 100x magnification using a QImaging MicroPublisher 3.3 RTV
attached to a Nikon Eclipse TE2000-U inverted microscope (Fig. S3A). From images, we measured two indices of bone remodeling: density of secondary osteons (Fig. S3B) and density of osteocytes (Fig. S3C) (after Hedgecock et al. 2007). We compared density of secondary osteons and osteocytes between the early and late ossifying regions of the mandible using a Wilcoxon two-sample test (Sokal and Rohlf 1995).

**DATA ANALYSIS**

*Mandible Size and Shape 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 1990). A principal component analysis (PCA) of landmark configurations (Procrustes coordinates) was used to summarize the major axes of mandible shape variation for early and late maturing regions. The relationship between mandible bite force and mandible form was examined by regressing bite force on centroid size and the first principal component (PC) of shape for both regions of the mandible.

*Effects of Muscle Stress on Mandible Growth, Development, and Remodeling*— To examine the relationship between muscle function and mandible development, we evaluated concordance of landmark displacements associated with muscle function and
mandible development (see discussion of FA in ‘Skull Morphology’ for assessment of
developmental variation) by calculating the angle between the first eigenvector of each
effect. The vector angle between two PCs was calculated as:

$$\Theta = \arccos \left( \frac{A \cdot B}{|A| \cdot |B|} \right)$$

where A and B are vectors containing the PC eigenvectors of each shape coordinate, and
|A| and |B| are the length of A and B; a more acute angle indicates greater similarity.

Landmark configurations among individuals, due to developmental variation, due to
muscle function, and due to measurement error were partitioned with ANOVA (Goodall
1991). We calculated vector angles (means ± bootstrapped standard deviations) for the
first PC of developmental variation and each muscle. Bootstrapped standard deviations
were calculated by sampling with replacement x- and y-coordinates (n = 1000 iterations)
and recalculating vector angles between the first PC of each effect. We evaluated the
early and late ossifying regions separately by calculating vectors containing only
landmarks within each region (Fig. 1). The effect of muscle function on mandible size
was measured with a multiple regression with centroid size as a dependent variable and
muscle function (for the M. digastricus, M. masseter, and M. temporalis) as the
independent variables. 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 errors in normal development (i.e., the accumulation of FA
(Hallgrimsson 1998; Hallgrimsson 1999)) in early and late ossifying regions of the
mandible. Shape variance among individuals and associated with 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.

**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 1985). Variation in mandible shape was partitioned among effects of species, individual identity, and measurement error using ANOVA (Goodall 1991), where individual identity was nested within species and entered as a random effect, and repeated measure was entered as a fixed 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 products for early and late maturing regions separately (as above). Variances were rescaled to adjust for size differences between the regions by multiplying the multivariate variance by the mean centroid size for the appropriate region. We compared directionality of epigenetically-induced variation and interspecific divergence in shape of the early and late ossifying regions of the mandible by assessing
concordance of landmark displacements associated with divergence across species and muscle function within species. We calculated the angles (means ± bootstrapped standard deviations) between the first eigenvector of each effect. We evaluated landmark displacements due to each effect in the early and late maturing regions separately by calculating vectors containing only landmarks within each region (see Fig. 1).

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REFERENCES


Table 1. Effect of muscle function on size of early and late ossifying mandibular regions.

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 (Fig. S1) are limited to the late ossifying region only. Shown are multiple regressions of mandible size on muscle function. $b_{ST}$ is the standardized partial regression coefficient. * P < 0.05, ** P < 0.01.

<table>
<thead>
<tr>
<th>Ossification Timing</th>
<th>$M.\ digastricus$</th>
<th>$M.\ masseter$</th>
<th>$M.\ temporalis$</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>$b_{ST}$</td>
<td>t</td>
<td>$b_{ST}$</td>
</tr>
<tr>
<td>Early</td>
<td>0.01</td>
<td>-0.06</td>
<td>0.07</td>
</tr>
<tr>
<td>Late</td>
<td>0.41**</td>
<td>2.04**</td>
<td>-0.44**</td>
</tr>
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</table>
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 towards the articulation with the skull. The early ossifying region is described by landmarks 11-19, and the late ossifying region – by landmarks 1-10 (see ‘Skull Morphology’ in Supporting Online Material). B) Attachment location of muscles *M. temporalis* (1), *M. masseter* (2), and *M. digastricus* (3) on the shrew mandible (for expanded functional description of the mandible see Fig. S1). C) Bite force was measured using bite plates attached to Kistler force transducer and charge amplifier for 26 live-captured *Sorex monticolus*. 
Figure 2. Muscle activity due to foraging strongly influenced development and associated function of the resulting morphology in the late maturing region of the mandible. (A) Developmental variation in mandible shape, measured covariation among landmarks in FA (see ‘Skull Morphology’ in Supporting Online Material), was highly concordant with shape variation due to activity of *M. digastricus* (*t* = 2.26, *P* < 0.05) and *M. temporalis* (*t* = 7.11, *P* < 0.001) in the late but not early ossifying region of the mandible. The developmental effects of *M. masseter* did not differ between the early and late ossifying regions of the mandible (*t* = 0.15, *P* = 0.88). Variation in mandible shape (direction and magnitude of landmark variation) was measured the first eigenvector of shape variation due to each effect for landmarks in the early and late ossifying regions separately (Fig. 1A). Shown are angles (mean ± bootstrapped standard deviations) between the first eigenvectors describing mandible shape for each effect. Smaller angles indicate greater developmental effect of muscle activity. (B) Developmental accumulation of variation differed between the early and late ossifying regions. The degree of shape variation among individuals in 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 FA, was significantly lower in the late maturing region of the mandible (variance: early = 0.05, late = 0.02, *t* = 6.98, *P* 0.01). Neither variation in size (*C bst* = -0.27, *P* = 0.2) nor shape (*D; bst* = 0.04, *P* = 0.85) of the early ossifying region contributed to bite force. On the contrary, in the late ossifying region, both size and shape variation contributed to
differences in bite force (E, size: \( bst = 0.39, P < 0.06 \); F, shape: \( bst = -0.49, P < 0.02 \)).

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 variation in landmarks contained in the early and late ossifying regions separately (Fig. 1A).
Fig. 3. Interspecific diversification in form of the early and late maturing regions of the mandible. (A) Across species, mandible size variation (plotted as the interspecific coefficients of variation, CV) was higher in the late ossifying region compared to the early ossifying regions (Levene’s test, $F = 10.5, P < 0.01$). (B) Interspecific shape variation (measured as the means square variance of landmark variation among species) did not differ between the early and late ossifying regions ($t = 0.17, P = 0.86$). The directionality of shape diversification was highly concordant with intraspecific variation in mandible shape due to muscle activity in the late but not early ossifying region of the mandible ($t = -2.92, P < 0.05$). Shown are angles (mean ± bootstrapped standard deviations). Concordance is the angle between the first eigenvectors describing mandible shape for each effect. Smaller angles indicate higher similarity in direction of variation between effects.
Figure S1. Distribution of epigenetic inputs due to foraging in the skull and mandible of montane shrew Sorex monticolus. A) The attachment location of *M. temporalis* on the mandible is outlined in black and the arrows indicate the direction of muscle contraction and points of muscles insertion on the skull. This is the largest masticatory muscle and it exerts the major crushing force on prey items (Dötsch 1994). B) Contraction of all muscles (A: *M. temporalis*, C: *M. masseter*, and D: *M. digastricus*) generates force on the mandibular articulation resulting in epigenetic stimulation on the mandible joint (condyle, red arrows). Mandible movements due to muscle action result in joint reaction forces, or the force of the mandible joint against the skull articulation (Herrel et al. 1998b; Hiiemae 2000). C) Attachment locations of the *M. masseter* on the mandible are outlined in blue and arrows indicate direction of contraction and the point of *M. masseter* insertion on the skull. The *M. masseter* contributes to crushing force and is the primary muscle used for lateral movements of prey grinding (Dötsch 1994). D) Attachment location of the *M. digastricus* on the mandible is outlined in green and the arrow indicates direction of muscle contraction and the point of *M. digastricus* insertion on the skull. The *M. digastricus* is the primary muscle of jaw opening (Dötsch 1985; Dötsch 1994). Action of the *M. digastricus* is an important determinant of gape angle and of bite force (Fig. S2A) (Carraway and Verts 1994; Young et al. 2007). E) The arrow indicates the major bite point of the jaw (Dötsch 1985). Because capture and mastication of prey items exerts force on the dentition (Dötsch 1985; Herrel et al. 1998b), action of the *M. temporalis* and *M. masseter* associated with crushing, handling, and grinding of prey items not only exerts force on the muscle attachment location on the mandible, but also results in
mechanical stimulation of the dentition and associated mandibular region (Herrel et al. 1998b). The forces of these epigenetic inputs are proportional to the action of the muscles.
Figure S2. Contribution of muscle function to empirically measured bite force. Function of muscles *M. digastricus* and *M. masseter* influenced individual bite force (A: *M. digastricus*: bst = 0.41, P < 0.05; B: *M. masseter*: bst = 0.53, P < 0.01; D: *M. temporalis*: bst = 0.34, P = 0.09). Muscle function was measured 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 (after Herzog 1994; after Herrel et al. 1998a).
Figure S3. Bone remodeling in the early and late ossifying regions of the Sorex monticolus mandible. A) Sagittal section of the S. monticolus mandible stained with hematoxylin (blue) and eosin (red). Stained sections were used to identify and measure density of secondary osteons (B) and osteocytes (C). Because secondary osteons develop from the remodeling of existing bone (Young et al. 2006) and osteocytes receive mechanical inputs and initiate bone remodeling (Noble et al. 2003), density of these two factors can be used as an index of the degree of bone remodeling (Hedgecock et al. 2007). Early and late ossifying regions of the mandible show equivalent levels of bone remodeling; density of secondary osteons densities (D: $U = 141$, $P = 0.15$) and osteocytes (E: $U = 202$, $P = 0.98$) did not differ between the two regions. Shown are means ± 1SE. Secondary osteons were identified by the presence of a cementing line marking the boundary of bone resorption (B, arrow). Osteocytes were identified by cell morphology (C, arrow).