Evolution of ontogeny: linking epigenetic remodeling and genetic adaptation in skeletal structures

Rebecca L. Young[1] and Alexander V. Badyaev

Department of Ecology and Evolutionary Biology, University of Arizona, Tucson, AZ 85721 USA

Synopsis 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; Hermisson 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 Rodríguez-Trelles 2004; Rodríguez-Trelles et al. 2005; Yanai et al. 2006). Environmentally induced recruitment of this "hidden" variation may facilitate the generation of

From the symposium "Ecological Dimorphisms in Vertebrates: Proximate and Ultimate Causes" presented at the annual meeting of the Society for Integrative and Comparative Biology, January 3–7, 2007, at Phoenix, Arizona.

[1]E-mail: rlyoung@email.arizona.edu

Integrative and Comparative Biology, volume 47, number 2, pp. 234–244
doi:10.1093/icb/icm025
Advanced Access publication May 22, 2007

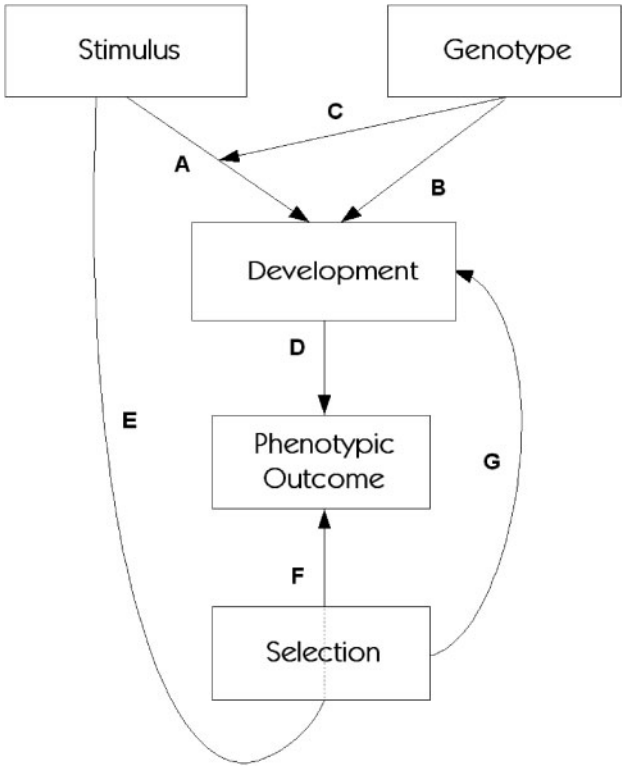

Fig. 1 Conceptual outline of the generation of phenotypic variants by changes in development. Development is influenced by a combination of epigenetic (**A**) and genetic (**B**) effects. (**C**) Sensitivity and response of development to epigenetic stimuli depend on both the strength of the stimulus 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.

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

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

Character	Role of BMPs	References
Morphological innovation		
Turtle carapace	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.	(Cebra-Thomas et al. 2005)
Bat wing	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.	(Sears et al. 2006)
Adaptation of existing skeletal structures		
Cichlid jaws	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.	(Terai et al. 2002; Albertson et al. 2005; Albertson and Kocher 2006)
Bird bills	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.	(Abzhanov et al. 2004; Wu et al. 2004, 2006)

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) and hard tissues during developmental vascularization and innervation (Warrell and Taylor 1979; Lanyon 1984; Herring 1993; Thorogood 1993).

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

Table 2 Evidence for sensitivity of genetic pathways of bone formation to epigenetic signals

Gene	Description of effect	References
Growth		
BMP-4	Tensile stress induced sustained upregulation of BMP-4 in mouse cranial suture zones.	(Ikegame et al. 2001; Mao and Nah 2004)
Ihh and BMP-2 and 4	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.	(Wu et al. 2001; Mao and Nah 2004)
FGF-2	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.	(Fong et al. 2003; Warren et al. 2003)
Remodeling		
BMP 3	Tension applied to leg bones of rats resulted in downregulation of BMP-3.	(Aspenberg et al. 2000)
Repair		
BMP-2, 4, and 6	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.	(Sato et al. 1999)
BMP-4	Upregulation of BMP-4 in tissues surrounding an artificial fracture.	(Nakase et al. 1994)

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 (know 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 pro-domain 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 non-functional 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

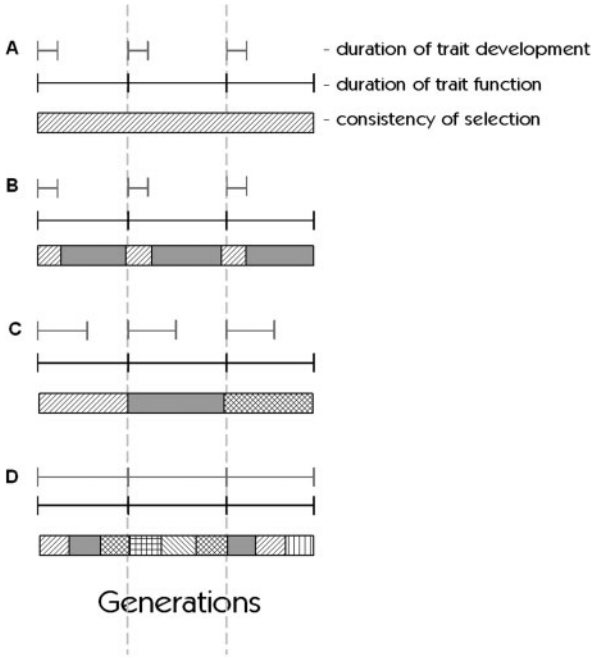

Fig. 2 Conceptual illustration of overlap between trait development and environmental exposure (e.g., through trait function) under distinct scenarios of within-generation reliability and trans-generational predictability of selection. (**A**) Selection is consistent within and across generations favoring 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.

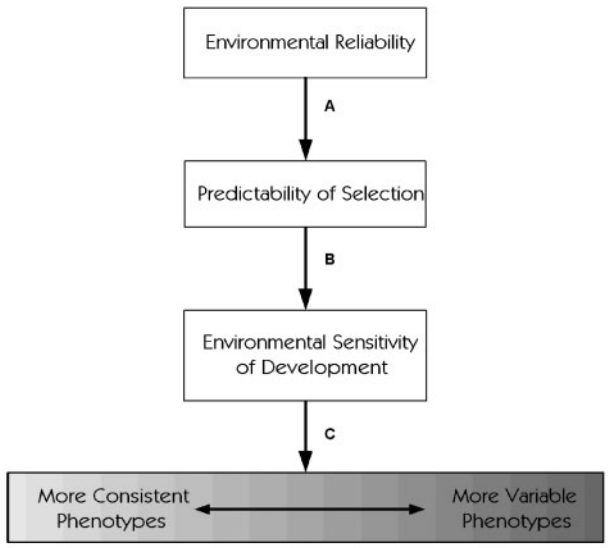

Fig. 3 Conceptual outline of evolutionary change in environmental sensitivity of development. (**A**) Reliability of the environment within and across generations determines the predictability of selection. (**B**) Predictability of selection determines the adaptive sensitivity to environmental variation during development by reliably favoring development of (**C**) a consistent phenotype or variable phenotypes.

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 remains 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.

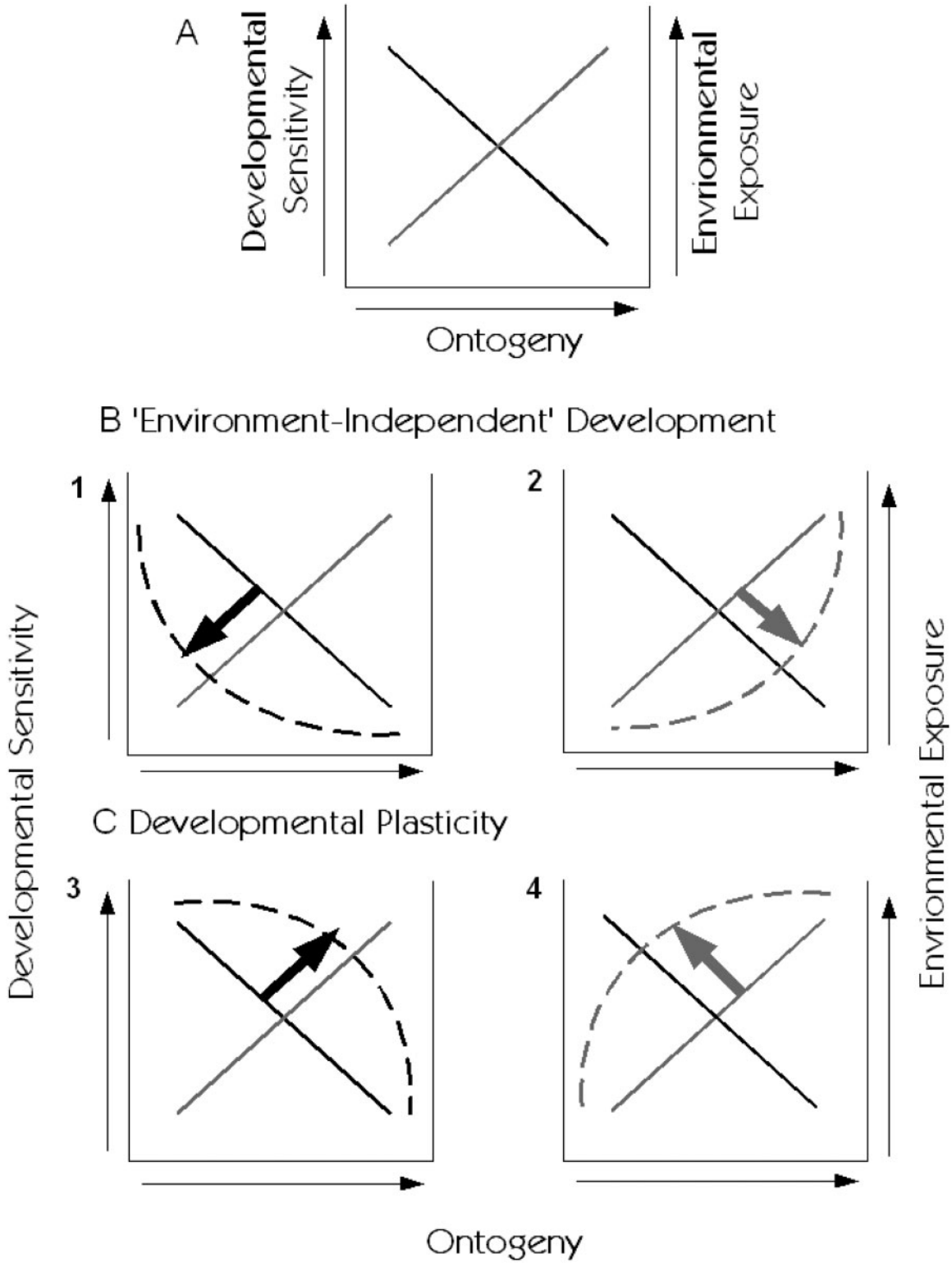

Fig. 4 Mechanism for evolutionary change in environmentally induced phenotypes. (**A**) Throughout ontogeny, sensitivity of development to environmental signal decreases (black line), whereas exposure to unpredictable environmental signals increases (gray line). (**B**) Increased developmental buffering 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.

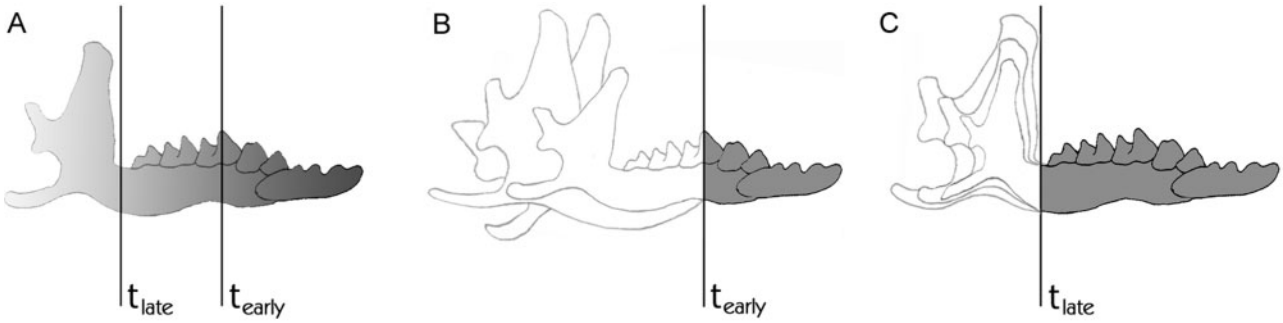

Fig. 5 Illustration of the influence of ontogenetic stage on response to external stimuli in the soricid shrew mandible. (**A**) Mandible tissues vary in ossification sequence; dark shading indicates earlier ossification. (**B**) External stress at early stages of ossification generates novel and variable phenotypes. (**C**) External stress at later stages of ossification results in channeled developmental variation resulting in similar patterns of variation among resulting phenotypes.

Acknowledgments

The authors are grateful to H. Heatwole and three anonymous reviewers for extensive comments on previous versions of this article and constructive suggestions. The authors also thank D. Acevedo Seaman, R. Duckworth, E. Landeen, K. Oh, J. Rutkowska, and E. Snell-Rood for discussions, and S. E. Vincent, S. P. Lailvaux, A. Herrel, and E. Taylor for the invitation to contribute to this symposium. This study was funded in part by the National Science Foundation grant (DEB-0608356) to R.L.Y. and by the David and Lucille Packard Fellowship to A.V.B.

References

Abzhanov A, Protas M, Grant BR, Grant PR, Tabin CJ. 2004. *BMP4* and morphological variation of beaks in Darwin's finches. Science 305:1462–5.

Albertson RC, Kocher TD. 2006. Genetic and developmental basis of cichlid trophic diversity. Heredity 97:211–21.

Albertson RC, Streelman JT, Kocher TD, Yelick PC. 2005. Integration and evolution of the cichlid mandible: the molecular basis of alternate feeding strategies. PNAS 102:16287–92.

Amundson R. 2005. The changing role of the embryo in evolutionary thought: roots of evo-devo. Cambridge: Cambridge University Press.

Anderson HC, Hodges PT, Aguilera XM, Missana L, Moylan PE. 2000. Bone morphogenetic protein (BMP) localization in developing human and rat growth plate, metaphysis, epiphysis, and articular cartilage. J Histochem Cytochem 48:1493–502.

Archer CW, Buxton P, Hall BK, Francis-West P. 2006. Mechanical regulation of secondary chondrogenesis. Biorheology 43:355–70.

Aspenberg P, Basic N, Tägil M, Vukicevic S. 2000. Reduced expression of BMP-3 due to mechanical loading. Acta Ortho Scand 71:558–62.

Atchley WR. 1993. Genetic and developmental aspects of variability in the mammalian mandible. In: Hanken J, Hall BK, editors. The skull. Chicago, IL: University of Chicago Press. p 207–47.

Atchley WR, Hall BK. 1991. A model for development and evolution of complex morphological structures. Biol Rev 66:101–57.

Atchley WR, Logsdon T, Cowley DE, Eisen EJ. 1991. Uterine effects, epigenetics, and postnatal skeletal development in the mouse. Evolution 45:891–909.

Badyaev AV. 2005a. Role of stress in evolution: from individual adaptability to evolutionary adaptation. In: Hallgrímsson B, Hall BK, editors. Variation. San Diego, CA: Elsevier Academic Press. p 277–302.

Badyaev AV. 2005b. Stress-induced variation in evolution: from behavioral plasticity to genetic assimilation. Proc Roy Soc Lond 272:877–86.

Badyaev AV, Foresman KR. 2004. Evolution of morphological integration: I. Functional units channel stress-induced variation in shrew mandibles. Am Nat 163:868–79.

Badyaev AV, Foresman KR, Young RL. 2005. Evolution of morphological integration: Developmental accommodation of stress-induced variation. Am Nat 166:382–95.

Baldwin JM. 1896. A new factor in evolution. Am Nat 30:441–51, 536–53.

Bergman A, Siegal ML. 2003. Evolutionary capacitance as a general feature of complex gene networks. Nature 424:549–52.

Bryan PN. 2002. Prodomains and protein folding catalysis. Chem Rev 102:4805–15.

Carroll RL. 2002. Evolution of the capacity to evolve. J Evol Biol 15:911–21.

Carroll SB. 2001. Chance and necessity: the evolution of morphological complexity and diversity. Nature 409:1102–09.

Carroll SB, Grenier JK, Weatherbee SD. 2001. From DNA to diversity: molecular genetics and the evolution of animal design. Oxford: Blackwell.

Cebra-Thomas J, Tan F, Sistla S, Estes E, Bender G, Kim C, Riccio P, Gilbert SF. 2005. How the turtle forms its shell: a paracrine hypothesis of carapace formation. J Exp Zool 304B:558–69.

Chen W, Zhao M, Mundy GR. 2004. Bone morphogenetic proteins. Growth factors 22:233–41.

Davidson EH. 2001. Genomic regulatory systems. San Diego: Academic Press.

de Jong G. 1999. Unpredictable selection in a structured population leads to local genetic differentiation in evolved reaction norms. J Evol Biol 12:839–51.

de Jong G, Gavrilets S. 2000. Maintenance of genetic variation in phenotypic plasticity: the role of environmental variation. Genet Res 76:295–304.

Eldredge N, Gould SJ. 1972. Punctuated equilibria: an alternative to phyletic gradualism. In: Schopf TJM, editor. Models in paleobiology. San Francisco: Freeman, Cooper and Company. p 82–115.

Emerson BM. 2003. Specificity of gene regulation. Cell 109:267–70.

Fong KD, et al. 2003. Equibiaxial tensile strain affects calvarial osteoblast biology. J Craniofacial Surg 14:348–55.

Frost HM. 1987. Bone 'mass' and the 'mechanostat': A proposal. The anatomical record 219:1–9.

Gerhart J, Kirschner M. 1997. Cells, embryos, and evolution: toward a cellular and developmental understanding of phenotypic variation and evolutionary adaptability. Malden, MA: Blackwell.

Gluckman PD, Hanson MA, Beedle AS. 2007. Early life events and their consequences for later disease: a life history and evolutionary perspective. Am J Human Biol 19:1–19.

Goldring MB, Tsuchimochi K, Ijiri K. 2006. The control of chondrogenesis. J Cell Biochem 97:33–44.

Gould SJ, Eldredge N. 1993. Punctuated equilibrium comes of age. Nature 366:223–7.

Heaney R. 1995. Bone mass, the mechanostat, and ethnic differences. J Clin Endocrinol Metab 80:2289–90.

Hermisson J, Wagner GP. 2004. The population genetic theory of hidden variation and genetic robustness. Genetics 168:2271–84.

Herring SW. 1993. Epigenetic and functional influences on skull growth. In: Hanken J, Hall BK, editors. The skull. Chicago: University of Chicago Press. p 153–206.

Herring SW, Lakars TC. 1981. Craniofacial development in the absence of muscle-contraction. J Craniofacial Genet Dev Biol 1:341–57.

Hino J, Kangawa K, Matsuo H, Nohno T, Nishimatsu S. 2004. Bone morphogenetic protein-3 family members and their biological functions. Front Biosci 9:1520–9.

Hogan BLM. 1996. Bone morphogenetic proteins: multifunctional regulators of vertebrate development. Genes Dev 10:1580–94.

Huiskes R. 2000. If bone is the answer, then what is the question? J Anat 197:145–56.

Ikegame M, Ishibashi O, Yoshizawa T, Shimomura J, Komori T, Ozawa H, Kawashima H. 2001. Tensile stress induces bone morphogenetic protein 4 in preosteoblastic and fibroblastic cells, which later differentiate into osteoblasts leading to osteogenesis in the mouse calvariae in organ culture. J Bone Miner Res 16:24–32.

Lanyon LE. 1984. Functional strain as a determinant for bone remodeling. Calc Tissue Int 36:56–61.

Larsen E. 2005. Developmental origins of variation. In: Hallgrímsson B, Hall BK, editors. Variation. San Diego, CA: Elsevier Academic Press. p 113–29.

Lauder GV. 1981. Form and function: structural analysis in evolutionary morphology. Paleobiology 7:430–42.

Lobe SL, Bernstein MC, German RZ. 2006. Life-long protein malnutrition in the rat (*Rattus norvegicus*) results in altered patterns of craniofacial growth and smaller individuals. J Anat 208:795–812.

Mao JJ, Nah H-D. 2004. Growth and development: hereditary and mechanical modulations. Am J Orthod Dentofacial Orthop 125:676–89.

Meyers LA. 2005. Constraint on variation from genotype through phenotype to fitness. In: Hallgrímsson B, Hall BK, editors. Variation. San Diego, CA: Elsevier Academic Press.

Moore SW. 2003. Scrambled eggs: Mechanical forces as ecological factors in early development. Evol Dev 5:61–6.

Müller GB. 2003. Embryonic motility: environmental influences and evolutionary innovation. Evol Dev 5:56–60.

Nakase T, et al. 1994. Transient and localized expression of bone morphogenetic protein 4 messenger RNA during fracture healing. J Bone Miner Res 9:651–9.

Newman SA, Müller GB. 2001. Epigenetic mechanisms of character origination. In: Wagner GP, editor. The character concept in evolutionary biology. San Diego, CA: Academic Press.

Newman SA, Müller GB. 2005. Origination and innovation in the vertebrate limb skeleton: an epigenetic perspective. J Exp Zool 304B:593–609.

Oyama S. 2000. The ontogeny of information. Durham, NC: Duke University Press.

Parfitt AM. 1997. Genetic effects on bone mass and turnover-relevance to black/white differences. J Am Coll Nutr 16:325–33.

Piersma T, Drent J. 2003. Phenotypic flexibility and the evolution of organismal design. Trends Ecol Evol 18:228–33.

Pigliucci M. 2005. Evolution of phenotypic plasticity: Where are we going now? Trends Ecol Evol 20:481–6.

Pigliucci M, Murren CJ, Schlichting CD. 2006. Phenotypic plasticity and evolution by genetic assimilation. J Exp Biol 209:2362–7.

Pizette S, Niswander L. 2000. Bmps are required at two steps of limb chondrogenesis: formation of prechondrogenic condensations and their differentiation into chondrocytes. Dev Biol 219:237–49.

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

Rapp RA, Wendel JF. 2005. Epigenetics and plant evolution. New Phytol 168:81–91.

Rauch F, Schoenau E. 2001. The developing bone: slave or master of its cells and molecules? Pediatr Res 50:309–14.

Rice SH. 2004. Developmental associations between traits: covariance and beyond. Genetics 166:513–26.

Rodríguez-Trelles F. 2004. Evolutionary genetics: transcriptome evolution – much ado about nothing? Heredity 93:405–6.

Rodríguez-Trelles F, Tarrío R, Ayala FJ. 2005. Is ectopic expression caused by deregulatory mutations or due to gene-regulation leaks with evolutionary potential? BioEssays 27:592–601.

Sato M, Ochi T, Nakase T, Hirota S, Kitamura Y, Nomura S, Yasui N. 1999. Mechanical tension-stress induces expression of bone morphogenetic protein BMP-2 and BMP-4, but not BMP-6, BMP-7, and GDF-5 mRNA, during distraction osteogenesis. J Bone Miner Res 14:1084–95.

Scheiner SM. 1993. Genetics and evolution of phenotypic plasticity. Ann Rev Ecol Syst 24:35–68.

Scheiner SM. 2002. Selection experiments and the study of phenotypic plasticity. J Evol Biol 15:889–98.

Schlichting CD. 2003. Origins of differentiation via phenotypic plasticity. Evol Dev 5:98–105.

Schlichting CD, Pigliucci M. 1998. Phenotypic evolution. Sunderland: Sinauer Associates.

Schmalhausen II. 1949. Factors of evolution. Philadelphia: Blakiston.

Scott MP. 1994. Intimations of a creature. Cell 79:1121–4.

Sears KE, Behringer RR, Rasweiler JJ, IV, Niswander LA. 2006. Development of bat flight: morphologic and molecular evolution of bat wing digits. PNAS 103:6581–6.

Siegal ML, Bergman A. 2002. Waddington's canalization revisited: developmental stability and evolution. PNAS 99:10528–32.

Simpson GG. 1953. The Baldwin effect. Evolution 7:110–7.

Skerry T. 2000. Biomechanical influences on skeletal growth and development. In: O'Higgins P, Cohn MJ, editors. Development, growth and evolution: implications for the study of the hominid skeleton. New York: Academic Press. p 29–39.

Smith MM, Hall BK. 1990. Development and evolutionary origins of vertebrate skeletogenic and odontogenic tissues. Biol Rev Camb Philos Soc 65:277–374.

Stern DL. 2000. Evolutionary developmental biology and the problem of variation. Evolution 54:1079–91.

Terai Y, Morikawa N, Okada N. 2002. The evolution of the pro-domain of bone morphogenetic protein 4 (BMP4) in an explosively speciated lineage of east African cichlid fishes. Mol Biol Evol 19:1628–32.

Thorogood P. 1993. Differentiation and morphogenesis of cranial skeletal tissues. In: Hanken J, Hall BK, editors. The skull. Chicago: The University of Chicago Press. p 112–50.

Tsuji K, Bandyopadhyay A, Harfe BD, Cox K, Kakar S, Gerstenfeld L, Einhorn T, Tabin CJ, Rosen V. 2006. Bmp2 activity, although dispensable for bone formation, is required for the initiation of fracture healing. Nat Genet 38:1424–9.

Tsumaki N, Yoshikawa H. 2005. The role of bone morphogenetic proteins in endochondral bone formation. Cytokine Growth Factor Rev 16:279–85.

Urist MR. 1997. Bone morphogenetic protein: The molecularization of skeletal system development. J Bone Miner Res 12:343–6.

Wagner GP, Mezey JG. 2004. The role of genetic architecture constraints for the origin of variational modularity. In: Schlosser G, Wagner GP, editors. Modularity in Development and Evolution. Chicago, IL: Chicago University Press. p 338–58.

Warrell E, Taylor JF. 1979. The role of periosteal tension in the growth of long bones. J Anat 128:179–84.

Warren SM, Brunet LJ, Harland RM, Economides AN, Longaker MT. 2003. The BMP antagonist noggin regulates cranial suture fusion. Nature 422:625–9.

West-Eberhard MJ. 2003. Developmental plasticity and evolution. Oxford: Oxford University Press.

Wilkins AS. 2002. The evolution of developmental pathways. Sunderland, MA: Sinauer Associates, Inc.

Wu P, Jiang TX, Shen JY, Widelitz RB, Chuong CM. 2006. Morphoregulation of avian beaks: comparative mapping of growth zone activities and morphological evolution. Dev Dynam 235:1400–12.

Wu P, Jiang T-X, Suksaweang S, Widelitz RB, Chuong C-M. 2004. Molecular shaping of the beak. Science 305:1465–6.

Wu Q, Zhang Y, Chen Q. 2001. Indian hedgehog is an essential component of mechanotransduction complex to stimulate chondrocyte proliferation. J Biol Chem 276:35290–6.

Wutzl A, et al. 2006. Bone morphogenetic proteins 5 and 6 stimulate osteoclast generation. J Biomed Mater Res 77A:75–83.

Yampolsky LY, Stoltzfus A. 2001. Bias in the introduction of variation as an orienting factor in evolution. Evol Dev 3:73–83.

Yanai I, Korbel JO, Boue S, McWeeney SK, Bork P, Lercher MJ. 2006. Similar gene expression profiles do not imply similar tissue functions. Trends Gen 22:132–8.

Yoon BS, Lyons KM. 2004. Multiple functions of BMPs in chondrogenesis. J Cell Biochem 93:93–103.

Young RL, Badyaev AV. 2006. Evolutionary persistence of phenotypic integration: influence of developmental and functional relationships on complex trait evolution. Evolution 60:1291–9.