

Evo-devo and constraints on selection

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Developmental bias, or genetic channeling, can influence the tempo and direction of evolution and, thus, become reflected in patterns of biodiversity. Twenty years ago, this notion rested on armchair descriptions of potential constraints on evolution. Now, a broad evo-devo approach involving both evolutionary and developmental genetics provides experimental analysis of such bias, revealing how shared genetic or developmental pathways among morphological traits contribute to the evolution of complexity and diversity, and that developmental bias itself evolves, generating variation in evolvability. As I discuss here, it is now possible to determine the relative importance of natural selection and of genetical and developmental architecture as determinants of organic diversity, particularly for examples of adaptive radiations and parallel evolution.

A broad evo-devo and differences in morphology

There has always been interest in how developmental processes change in the evolution of morphologies. However, only during the past two decades have these issues been opened up to analysis through modern evolutionary developmental biology, or evo-devo [1]. Variation in morphology can now be mapped onto genetic variation via developmental mechanisms and the processes of pattern formation and morphogenesis [2]. This can be done for morphologies that are relevant in an ecological arena, such as interactions with predators or with potential mating partners. For example, whether different populations of stickleback fish have reduced pelvic spines depends on the expression of the *Pitx1* gene [3], and whether the wings of males of different species of fruit fly are patterned with an apical black spot is primarily the result of the expression pattern of the gene *yellow* [4]. Integrating such insights with experimental studies of natural selection and comparative analyses of morphology in particular lineages or clades will test between the relative importance of selection versus constraint. Although I discuss such studies in animals here, similar progress is also being made in plants [5].

Many differences in complex morphological traits are not the result of the presence or absence of particular genes but are based on changes in the mechanisms of gene regulation affecting when and where a gene is expressed [6–8]. Thus, there is a limited genetic tool kit and much of morphological diversity evolution is about old genes performing new tricks. Although existing genetic pathways can be co-opted and subsequently elaborated upon to do something different, and specific genes can take

on additional tasks at new times during development and in different tissues via gene duplication and divergence, *de novo* evolution of new pathways appears to be rare. Our increasing knowledge of development can help us to understand how these processes influence evolutionary change in morphology and how they are reflected in current patterns of diversity.

The evolution of allometry

Two related issues in biology now opening up to experimental analysis are how the processes that generate morphological variation bias evolution, and how patterns of allometric growth change during evolution. The impact of most illustrations of related species of animals reflects diversity in form arising from changes in the size of morphological features, such as appendages, relative to one another, and to overall body size. Although

Glossary

Absolute constraint: one that is impossible to break even over very long periods of time and, thus, of mutational input. A strict constraint, although not impossible to break, would require some form of evolutionary novelty to do so.

Convergent evolution (convergence): evolution of similar phenotypic features independently in different lineages, usually from different antecedent features, and assumed to involve different developmental pathways.

Developmental constraint: a bias on the production of various phenotypes caused by the structure, character, composition, or dynamics of the developmental system [20],[20]

External and internal factors: external refers to influences on the phenotype via the biotic and abiotic environment of the organism, especially natural selection, whereas internal refers to the processes of genetics, development and physiology, involved directly in making the phenotype and in generating variation in the phenotype.

Generative constraint: one that involves the internal processes that govern how the phenotype is generated. It can comprise some combination of genetic channeling and developmental bias.

Genetic channeling: leads to a trend in a set of traits towards evolution along a genetic line of least resistance that reflects the consequences of shared genetic pathways and genetic variation [24]. Similarly, a developmental line of least resistance could result from shared developmental pathways.[24]

Modularity: ability of individual parts of an organism, such as repeated pattern elements, limbs, or segments, to develop or evolve independently from one another; the ability of the regulation of development to be modulated independently in different tissues and stages. Different elements within a single module will lack individuality and the ability for independent evolution, but show independence from other modules.

Morphospace: hypothetical unconstrained phenotypic space available for occupancy by a particular morphology, usually within an evolutionary lineage.

Parallel evolution (parallelism): evolution of similar or identical features independently in related lineages, usually considered to be based on similar modifications of the same developmental pathways.

Pleiotropy: phenotypic effect of a gene on more than one trait or character that contributes to a genetic correlation between them. Combinations of pleiotropic effects can account for genetic channeling.

Relative constraint: effect of a bias in the generation of phenotypic variation, rather than of any strict limitation. Such a bias can affect evolutionary change, for example, because only a limited window of time is available for adaptation in a particular direction in response to a new environment. The basis of such constraints could lie solely in the standing genetic and developmental variation within populations.

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the tinkering on themes of form that results from changes in patterns of allometric growth is a major source of diversification [9–13], we know little about the underlying proximate and causal processes, or about how such internal and external factors interact with one another to yield the observed patterns of evolution.

In contrast to the variation in morphological traits among taxa, allometric relationships within species typically show little variation [14–16]. For example, eye stalk length within species of diopsid flies varies little relative to body length, but can differ dramatically among species [17,18]. How then can a comparative absence of variability within individual populations or species be reconciled with such disparity across taxa? Examining such specific issues about patterns of variation, as well as testing selection or constraint, requires a broad approach to evo-devo [19]. This will extend from experimental studies of how morphologies are made in model organisms, to comparative analyses of patterns of diversity within groups of related taxa, and will ultimately reveal why morphospace (see Glossary) is occupied by species in the way it is.

Evolutionary constraints and the generation of the phenotype

The topic of constraints and how they influence the dynamics of evolution has fascinated evolutionary biologists from long before the origin of *TREE*. Do constraints, other than the more obvious absolute or physical examples (e.g. rate of oxygen diffusion and insect body size), influence evolutionary patterns of change or stasis?

Developmental bias and genetic channeling

An early key review [20] established the crucial idea of developmental bias: do the properties of the developmental system bias the course of evolution? Following on from ideas about how genetic covariances among traits could influence the tempo and direction of evolution [21–23], Schluter [24] developed the influential notion of genetic channeling, which could result in the clustering of populations or species along lines or axes of least resistance. More case studies and experimental data are necessary to discover whether developmental bias and genetic channeling are just two sides of the same coin. Whether they are will have implications for our ability to apply the appropriate adjectives in descriptions of constraints on the evolution of morphology.

Perhaps a wider term, such as generative constraints, will be useful to encompass the roles of developmental bias and genetic channeling in the processes of generating evolutionarily relevant variation in the phenotype. However, thinking about developmental and genetical mechanisms (including those of epigenetics) might not cover all such generative constraints. For example, hormones coordinate the control of growth, as well as the maintenance of function once development has run its course [25]. Whereas the expression of genes controlling developmental decisions via cell–cell signaling pathways occurs within comparatively small populations of cells, hormones are long-distance signalling mechanisms, often

with multiple populations of target cells or tissues. There might be fundamental differences in the consequences of such properties for the evolution of complexity. For example, differential expression of a developmental gene in a particular tissue can be achieved independently of effects in other tissues, whereas, because many target tissues experience the same circulating hormone, uncoupling the effects in different tissues will usually necessitate changes in receptor expression or downstream targets. Thus, if different traits, especially life-history and behavioral traits, are modulated by the same physiological mechanism, this needs to be taken into account when considering their evolutionary dynamics [26,27]. Hormones, and their downstream targets, are of special significance in the control of growth patterns among different tissues and organs, and are likely to be crucial in the evolution of allometries [28–30].

Thus, any unitary framework for generative constraints or developmental bias will need to consider how the phenotype is built and maintained not only via the regulation of genes and epigenetic processes, but also via physiological mechanisms. Either a move is needed towards a wider view of what development encompasses, or comparative and evolutionary physiologists must become more involved in the broader initiatives of functional genomics and of integrating ecology within evo-devo [31–33].

The need for experimental analysis

Descriptions of potential constraints on evolution have been characterized by a lack of experimental analysis. This is illustrated by Raup's classic example of how mollusc species fill plots of potential morphological space for shell form as described by three parameters of growth [34]. Although such maps of species occurrence in morphospace generally emphasize how large parts of the potential space are empty, these descriptions are not sufficient to confirm the involvement of developmental bias or genetic channeling. However, this will change as experimental analyses place such descriptions in a more experimental framework that also examines genetical and developmental mechanisms and, thus, can begin to test between selection and constraint.

Absolute or strict constraints undoubtedly account for some boundaries in patterns of occupancy of morphological space. However, I consider that relative constraints or patterns of bias in the generation of phenotypic variation will prove more interesting and, debatably, more relevant to our understanding of the overall distribution of species in morphospace. A relative constraint or bias in the generation of phenotypic variation might channel evolutionary responses to new environments because of limitations arising from the combination of the time available for adaptation to occur and the evolvability of the traits involved. Specialization might also occur more rapidly along some line of least resistance that then makes alternative directions of evolution increasingly less likely [35]. Analyses of traits with shared genetics or development will explore the likelihood of adaptive evolution in different directions of morphospace.

How constrained are adaptive radiations?

It is therefore relevant to ask to what extent examples of adaptive radiation are shaped by natural selection alone rather than being compromised in some way by the processes that generate phenotypic variation [36]. Given that some evolutionary innovation has occurred or some new ecological opportunity has arisen, we can then ask whether the subsequent pulse of elaboration on such a new theme is directed only, or even primarily, by natural selection. Such issues have led to much debate and discussion with numerous examples of potential modes of constraints [37–41].

Even in a dramatic example of adaptive radiation, such as Darwin's finches [42,43], the ways in which variation in bill morphology has been generated might influence the morphologies found among the present-day species. In other words, even in the face of the intense natural selection known to occur, at least intermittently, in this system [44], the forms of bills and other morphological traits might be different from those currently observed if development worked in another way and was based on different genetic pathways and key control points [45]. Further exploration of such issues requires a focus on systems that can be analyzed at all levels, from genes to fitness effects.

Parallel evolution: selection or constraint?

Patterns of parallel evolution can provide even stronger illustrations of the need to distinguish explanations based on the similarity of natural selection from those involving developmental bias or genetic channeling. The species flocks of Haplochromine cichlids in the African lakes of the Rift Valley are enthralling examples of parallel evolution and adaptive radiation (Figure 1). There is a striking similarity in the forms of the modal trophic morphologies, from the body, head and fins to life-history and behavioral traits, that have evolved in each lake from different founder populations [46,47]. Are these ecomorphs a reflection of shared environments and modes of natural selection, or are they, at least in part, suites of traits that emerge because of the ways in which phenotypic variation is generated? In the latter case, the course of evolution will depend on the evolvabilities of the traits concerned and their interactions, as well as how these properties match up to the shapes of fitness curves and the demands of natural selection in different directions. The relative contributions of such intrinsic and extrinsic factors will remain unclear until more is known not only about functional morphology and how natural selection works for different case studies, but also about precisely how development maps phenotypic variation onto genetic variation in these examples [48–51]. Applying a broad evo-devo approach that combines experimental analyses using model species with comparative analyses based on phylogenetic reconstructions will provide new insights [52–54].

Thus, although natural selection in novel environments has no predetermined endpoints, examples of parallel evolution such as in the African cichlids suggest that the ways in which phenotypic variation is generated orchestrate adaptive evolution along certain trajectories.

Perhaps patterns of parallel morphological evolution given similar ecological opportunities are to be expected and that, given sufficient knowledge of developmental processes, they could be predictable. Such considerations about evolvability and the repeatability of evolution apply to macroevolutionary events and radiations through to adaptive microevolution in multiple populations of the same organism presented with a similar environmental challenge [1,55–59].

Molecular analyses of replicated asexual populations of *Escherichia coli* that had undergone experimental evolution over thousands of generations in a novel nutrient environment, revealed a high degree of parallel evolution with respect to the genes in which key mutations occurred [60,61]. Parallel evolution involving mutations in the protein-encoding sequences of the same genes is also being detected for the MC1R protein in several cases of melanism in mammals and birds [62,63], and for the region encoding the Oca2 protein responsible for albinism in multiple cavefish populations [64]. These proteins have a high degree of tissue and functional specificity, and the genes probably show few, if any, other phenotypic effects and, thus, have minimal pleiotropy. The phenotypic trait can then evolve comparatively independently of other traits.

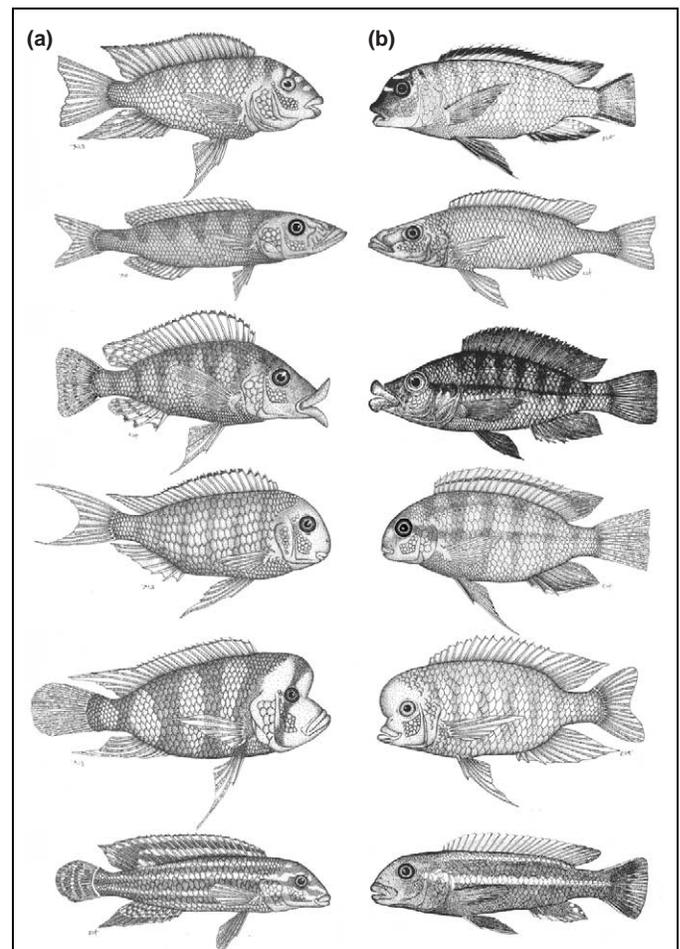


Figure 1. Parallel evolution of trophic morphologies (ecomorphs) in the species flocks of cichlid fishes from two African lakes: (a) Lake Tanganyika and (b) Lake Malawi. The species in each lake are more closely related to one another than to any species in another lake. Reproduced with permission from [47].

Such flexibility owing to the absence of any coupling is paralleled for more complex morphologies involving independent modules, each based on discrete genetic networks and patterning mechanisms [65–72]. By contrast, the developmental genes underlying animal forms are frequently expressed in many different cells and tissues, and at many different times during development. They are highly pleiotropic, and there is increasing evidence that evolutionary change tends to occur via extensive evolutionary tinkering in their complex regulatory apparatus, rather than in the encoding sequences themselves, and that particular sets of *cis*-regulatory elements are associated with specific modes of gene function [2,6–8].

Furthermore, although many genes are involved in making a complex trait, evolution of diversity within a clade probably tends to involve key regulatory genes with particular properties. Recent work on multiple gains and losses of dark pigment spots on the distal wing tips of males of some species in the *Drosophila* clade is demonstrating that the *yellow* gene acts as such an ‘evolutionary hot spot’ [5,73]. Two independent gains of wing spot in the *Drosophila* clade involve distinct *cis*-regulatory elements of this gene, and losses of the spot that involve the same regulatory element of *yellow* have also been identified. Thus, similar phenotypic changes can show some parallel evolution, not only in terms of the key developmental genes, but also at the level of their regulatory apparatus. The next decade should reveal how general such findings are for complex morphological traits in different clades.

Divergence among repeated structures

A fully integrative approach to testing how the filling of morphospace depends on natural selection and on constraint is materializing for complex morphological traits based on multiple copies of a particular element. One example is the evolution of mouse teeth, based on work ranging from developmental genetics with the laboratory mouse, to tracing the evolutionary history of changes in tooth morphology in the fossil record [74]. Furthermore, the experimental data are now such that robust simulation models are being developed of the evolution of developmental gene networks; in combination with the fossil record, this should reveal the evolution of the developmental processes involved [75].

Teeth, similar to butterfly wing eyespots, the bones of the vertebrate jaw or the limbs of an arthropod, illustrate the concept of modularity and, more particularly, the nested nature of pleiotropic gene interactions that can evolve within what were presumably single modules at their origin [67–72]. Many such complex sets of traits involve serial repeats or multiple copies of an element, and have their origins in sets of more similar structures; each element shares some common genetic and developmental architecture. But how do the different elements gain individuality and how does the evolution of complexity occur? Does the evolvability in such a system itself evolve in terms of responses to natural selection and with respect to the mechanisms that lead to some uncoupling of the development of different elements? Do these mechanisms, in turn, bias or channel the future paths of evolution for

the whole module, and thus become reflected as emergent properties in patterns of diversification in morphology?

Using artificial selection to examine evolutionary change

Artificial selection can be used not only to produce novel phenotypes for genetical and developmental analysis, but also to explore the potential for developmental bias and genetic channeling on evolutionary change. The degree of morphological flexibility in the evolution of repeated pattern elements is being examined using this tool for the wing eyespots of the tropical butterfly *Bicyclus anynana* [76,77].

Each individual eyespot along the wing margins is formed during the late larval and pupal stages around a central ‘signalling’ organizer or focus. The eyespots all share the same developmental process [78] and show parallel patterns of gene expression for several known developmental genes, including *Distal-less*, *engrailed*, *spalt* and *Notch* [79,80]. Selection on different traits of a single eyespot yields correlated responses in other eyespots, indicating positive genetic correlations for the same trait across eyespots (but not among traits). Furthermore, most single gene mutations influence all eyespots in a comparable manner. Artificial selection in different directions through morphological space for the pattern of the relative size of two eyespots on the same wing surface nevertheless reveals a remarkable degree of flexibility in their evolution [76]. Thus, 25 generations of selection yielded phenotypes for this pattern towards each corner of morphospace, either along the predicted line of least resistance reflecting the positive genetic covariance and developmental coupling of the two eyespots, or along the orthogonal axis to this that reflects changes in the size of the eyespots in opposite directions (i.e. one larger and the other smaller; Figure 2).

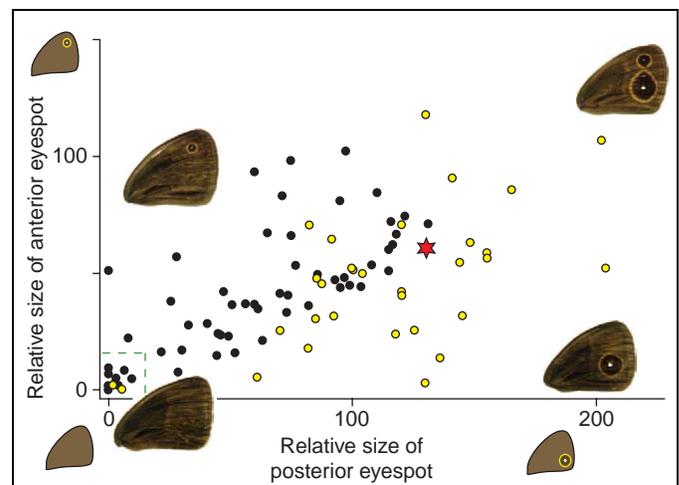


Figure 2. The occupancy of morphological space for the relative size of the two dorsal forewing eyespots of the butterfly *Bicyclus anynana* compared with the variation among species of this African genus and of the closely related Asian genus *Mycalesis*. The four images of the wing in each corner of the morphospace are representative examples of the wing pattern after 25 generations of artificial selection in *B. anynana* [76] towards each of these corners of morphospace, starting from the wild-type for this species as depicted by the star. The four wings are placed in roughly the correct position in the depicted trait space. Circles show the position of the mean patterns of the size of the same eyespots for different species of *Bicyclus* (closed symbols) and of *Mycalesis* (open symbols). The dotted square encloses species for which both eyespots are very small or absent, and frequently difficult to measure. Reproduced with permission from [19].

An ability to produce small undifferentiated spots of pigment centered midway between each of the pairs of veins towards the edge of the wings appears to have evolved as a novel patterning mechanism in early Lepidoptera [79]. Evolution has elaborated on this simple pattern, for example by adding the ability to form nested series of color rings centered around each spot. The flexibility of responses to artificial selection on eyespot size observed for a single stock of *B. anynana* probably reflects a long legacy of natural selection in favor of the formation of eyespots of different size across populations and species in different environments [77]. Natural selection with respect to predation [81,82] and to mate choice [83,84] has contributed to this evolution of diversity in size. The result is that, even in a single laboratory stock of one species, there is sufficient standing genetic variation for the mechanisms that specify eyespot size to enable extreme uncoupling over small numbers of generations. Thus, evolvability is high for this trait and any generative constraints are weak, although a residual bias in the system might still account for some clustering of related species along a genetic and developmental line of least resistance (Figure 2).

The pattern of different eyespots in *B. anynana* not only expresses variation in size but also in other traits, including color composition and shape. Artificial selection experiments on a single eyespot revealed similar heritabilities and responses to selection for color (the width of the outer gold ring relative to the inner black disc; Figure 2) but much lower for deviations from a circular shape [85,86]. Moreover, the developmental bases of these three traits differ: variation in eyespot size is primarily specified by the signal strength of each individual central eyespot organizer [87]; color composition is modulated by the response properties to these signals by the whole epidermal wing surface [85]; and tinkering with eyespot shape is achieved by changes in overall wing shape or in the spacing of the anterior–posterior rows of pigmented scale cells over the wing surface [88]. Experimental analyses can now be made of the consequences of these differing combinations of genetic variation and developmental properties for the evolvability of the different traits and of their patterns of diversity among species of this clade of butterflies [19]. Examinations of different species of *Bicyclus* and the closely related *Mycalesis* indicates that, although eyespot size varies in a highly flexible manner (Figure 2), eyespot color composition is characteristic of each species; some species have narrower gold rings whereas others have broader gold rings, but the eyespots on a single wing surface for any particular species all have a similar color pattern [19].

The form of different eyespots across a wing or of different teeth along a jawbone reflects patterns of allometric growth on a small scale relative to body size. However, the evolution of allometry is usually considered for more prominent body parts, such as different limbs. Evolutionary diversification in the patterns of relative growth of different appendages must involve uncoupling of traits that originally shared all of their genetic pathways and developmental mechanisms. Figure 3 illustrates pairs of butterfly species that are highly divergent either for the

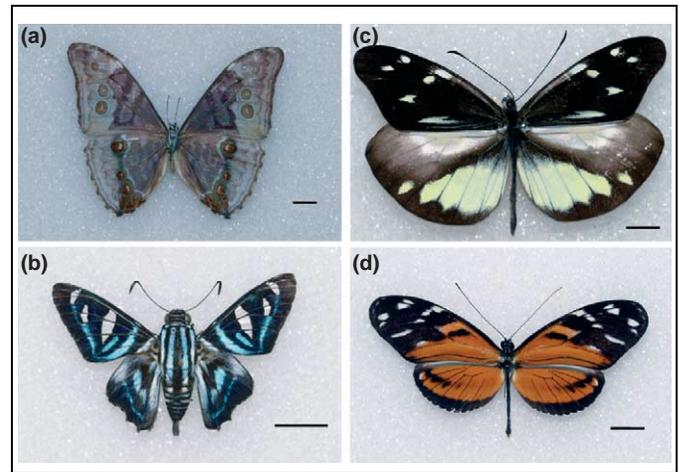


Figure 3. Examples of variation in allometry in butterflies [89,90]. Species illustrating divergence for the scaling relationship of wing size to body size or ‘wing loading’ (a,b) and forewing size to hindwing size (c,d) are shown. Scale bar = 1 cm. Images reproduced with permission from the Naturalis Museum in Leiden and H. Berkhoudt.

size of the forewings relative to the hindwings, or for the size of both pairs of wings relative to body size. Again, artificial selection in the model species *B. anynana* is exploring the potential flexibility in short-term responses in these scaling relationships [89,90].

Artificial selection resulted in divergence in the scaling relationship for wing size relative to body size [89] and for forewing to hindwing size [90], in each case producing novel morphologies relative to those in the base line population. Following selection, the populations with divergent scaling relationships for each pair of traits were crossed to produce single populations with wide phenotypic variance for each scaling relationship. These populations were then used to compare the mating success of males showing changed allometry with that of males of wild-type allometry. For each scaling relationship, the wild-type males had a substantially higher mating success than did either of the divergent phenotypes [89,90]. These studies provide support for the occurrence of strong stabilizing selection within species in specific environments, but also suggest the necessary evolvability to account for the evolution of diversity in scaling relationships for species in different environments. Thus, this analysis of allometry in *B. anynana* is beginning to examine how natural selection and generative constraints each contribute to specific patterns of variation and of evolutionary change.

Prospects

Integrative research on generative constraints in which genetic variation and developmental mechanisms are explored together with natural selection and the performance of phenotypes is necessary to progress beyond the level of ‘just-so stories’. For allometry, the extent of the evolvability of particular scaling relationships within model species can be examined in the context of the largely unknown mechanisms of genetics, development and physiology that control growth and modulate the size and form of different structures. Not only will such studies inform us about developmental bias in evolution, but they

will also reveal the bases of differences in evolvability among traits.

The integration of experimental work combining evolutionary genetics and (eco-)evo-devo with theoretical models for the evolution of particular developmental gene networks will become increasingly important in understanding the origins and consequences of evolvability. Placing such efforts for tractable experimental systems into the context of phylogenetic reconstructions of patterns of evolution for these morphologies will reveal much more about how the processes that generate variation in the phenotype have contributed to examples of radiations and parallel evolution. We will then know whether a substantial proportion of the variation in morphological diversity is explained by developmental bias, rather than by natural selection.

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