Micro-Evo-Devo

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Abstract

Micro-evo-devo ~~as~~ the study of genetic basis of developmentally mediated phenotypic variation, and the evolutionary forces that affect the fate of that variation within populations. Currently, there are few studies detailed enough to trace the short-term evolutionary events underlying changes in developmental processes, which leaves the general explanatory power of a micro-evo-devo approach unclear. One promising approach is to use artificial selection to directly cause evolution of developmentally mediated phenotypes and then infer the genetic and developmental underpinnings of that response. A second promising approach is to use a comparative approach in model systems such as sticklebacks that have repeatedly been challenged to adapt to similar environments in the recent past. Part of the reason that micro-evo-devo studies are still rare is an implicit assumption by some that the current variability of a population is irrelevant to long-term patterns, and that the vast majority of short-term changes in development are essentially random with respect to long-term trends. On the other hand, all major evolutionary transitions or trends must consist of micro-

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evolutionary changes. Emerging evidence from a variety of systems shows that long-term patterns can sometimes be retrodicted from variability within extant populations. Further development of micro-evo-devo approaches is needed to enable us to determine the generality of these results.

Keywords

Genotype-phenotype-fitness map · Micro-evo-devo · Evolve and resequence · Variability · Quantitative genetics

Introduction

Understanding the evolution of phenotypic diversity is an enduring goal of biology. Two complementary approaches are necessary to realize that goal: comparative and process-based. The comparative approach describes biological diversity among taxa from the genomic to the phenotypic level, and places that diversity in a phylogenetic context. The process-based approach investigates how traits that exemplify that diversity actually evolve within populations (see the chapter ▶ "A Process-Based Approach to the Study of Flower Morphological Variation").

The increasing importance of evolutionary developmental biology, or evo-devo, comes from the fact that much of that biological diversity is generated by developmental processes. The field of evo-devo arose from two nearly coincident developments in biology in the late 1970s and 1980s (Laubichler 2010). At this time, a number of evolutionary biologists reemphasized the explanation of the evolution of phenotypes rather than genes as their goal (see the chapter ▶ "The Role of Evo-Devo in the Extended Synthesis" for a different perspective on this shift). Nearly simultaneously, molecular developmental geneticists scored their first dramatic successes in identifying genes responsible for fundamental differences in development among taxa. Since then the core work in evo-devo has remained comparative, with a focus on identifying both the fundamental processes that underpin development in multiple taxa, and the underlying developmental and genetic basis of discrete differences among species. These approaches only indirectly inform us about the processes that generated developmental diversity.

This chapter briefly reviews the current status of the process-based approach in the evo-devo studies. Despite early calls for the process-based study of evo-devo (Stern 2000; Johnson and Porter 2001), such studies are still relatively uncommon. This leaves us uncertain whether the micro-level processes by which development must evolve influence the larger-scale events and patterns that can be studied using the comparative approach.

What Is Micro-Evo-Devo?

We define micro-evo-devo as the study of the nature and genetic basis of developmentally mediated phenotypic variation, and the evolutionary forces that affect the fate of that variation within populations. These twin emphases on the nature of phenotypic variation and on the interaction between that variation and natural selection, the genotype-phenotype-fitness map, are what make micro-evo-devo studies distinct from their parent disciplines, population, and quantitative genetics. The genotype-phenotype (GP) map (see the chapter ▶ "Genotype-Phenotype Map") is the biological machinery that turns a genotype into all the many phenotypes that an individual expresses during its lifetime (Lewontin 1974). By extending the map from phenotypes to fitness, one can in principle include all of the evolutionary forces that are hypothesized to give rise to properties of the GP map that generate the evolutionary properties of developmental systems, including evolvability (see the chapters ▶ "Evolvability" and ▶ "Variational Approaches to Evolvability: Short- and Long-Term Perspectives"), modularity (see the chapter ▶ "Modularity in Evo-Devo"), plasticity (see the chapters ▶ "Developmental Plasticity and Evolution" and ▶ "Eco-Evo-Devo"), robustness, etc. Today micro-evo-devo encompasses studies of mutation and standing variation that affect development, the responses of morphology, and the developmental processes that underlie it to natural and artificial selection, and the causes of natural selection on morphological traits. These approaches are related to and complement both the "devo-evo" approach to the study of how developmental processes shape the evolutionary process (see chapter ▶ "Developmental Evolutionary Biology (Devo-Evo)") and comparative studies of recently diverged populations or species where contemporary data on variation and selection is relevant to the divergence under study.

Traditional evo-devo can address the nature of the genetic and developmental differences that have given rise to biological diversity, but cannot directly answer questions about why that particular variation has been recruited to evolutionary divergence. For example, a finding that a particular difference in morphology is the result of changes in gene regulation tells us what must have evolved – the cis-regulatory elements of genes involved in development – but it does not tell us how or why they evolved that way. Are there alternative paths that evolution might have taken? If so, why weren't they? Only at the population level can we hope to study all the evolutionarily relevant variation and its phenotypic effects.

While evolutionary biologists have long realized that the study of the evolution of development must ultimately encompass the origin and fixation of genetic variants affecting development, it took longer for the new molecular tools of developmental genetics to become sufficiently inexpensive for population-level studies. Explicit micro-scale studies of evo-devo are still relatively uncommon (Nunes et al. 2013). While some of this relative neglect is based on the historical origins of evo-devo outlined above, we suspect that it is also due to an implicit assumption among at least some evo-devo practitioners that what happens at the micro scale is not very relevant to the differences among species and higher taxa (e.g., Milocco and Salazar-Ciudad 2020). The use of the term "micro" implies an overlap with the more general micro-

vs. macro-evolution distinction which concerns the relationship between evolution over short vs. long time scales or that within vs. among species. The skepticism that micro-evo-devo is relevant inherits some of aspects of the controversy over whether macro-evolution is just micro-evolution continued over a long time period (e.g., Gould 2002). Currently the connections between micro-evolution of development and the macro-pattern of diversity in development are unclear, as there are limited number of relevant studies. The key challenge is to either establish the relevance of micro-evo-devo to the larger issues in evolutionary developmental biology, or, alternatively, explain how larger-scale or longer-term processes render micro-level changes irrelevant.

Micro-Evo-Devo in Action

To illustrate the scope of micro-evo-devo studies, let's consider example studies that span the diversity of approaches that are possible.

The most direct micro approach is to observe the response of a population to selection on a developmentally relevant phenotype. Selection can be used in several contexts. Natural populations can frequently experience selection, but it is rarely clear what the actual target of selection might be. In artificial selection the investigator measures and selects the individuals chosen for breeding. Artificial selection experiments are thus ideal for testing hypotheses about the nature of the developmental response when a particular phenotypic change is selected for.

For example, Marchini and Rolian (2018) investigated the mechanism by which a mouse population responded to selection for tibia length, one of the long-bones that has diversified among mammals. After only 14 generations of artificial selection for increased tibia length standardized by body mass, they had increased average length by 9% and 14% in 2 replicate populations. During bone development, a scaffolding of cartilage is produced by chondrocytes at each end of the developing bone, undergoes proliferation and enlargement, and then that scaffolding is filled in by the osteoblasts that form ossified, mineralized bone. Based on this knowledge, Marchini and Rolian (2018) could identify four potential mechanisms for an evolved increase in tibia length: prolongation of the period of bone growth, increases in the number of dividing chondrocytes, increases in chondrocyte size, and increases in the proliferation rate during ossification. Analysis of mice at the end of the experiment showed that the long-limbed treatments had increased the number of chondrocytes, while no differences were found in the other three characteristics. In this population, evolution did not proceed by all possible means, but was focused on just one process. This is in contrast with the differences in growth between limbs in bats, which have elongated forelimbs, and jerboas, which have elongated hindlimbs. In these taxa, increases in chondrocyte size are important in the elongated body parts. This difference in mechanism may reflect the fact that the experimental mice were selected to increase the length of one limb, but not to change the relative growth of their limbs.

Other micro-evo-devo studies investigate the genetic changes underlying changes in development, rather than directly studying development. In an evolve-and-resequence (E&R) experiment, next-generation sequencing of selected populations identifies the genomic changes that distinguish the populations after selection. Changes that cannot be explained by drift are inferred to be due to selection. For example, Turner et al. (2011) selected replicate populations of *Drosophila melanogaster* to have larger or smaller body size for 100 generations. The genomic response was traceable to a large number of genomic regions. The regions that responded to selection were far more likely to code for genes involved in the gene ontology categories anatomical development, cell number, and metamorphosis. This suggests that the changes in size in this experiment were accomplished by small alterations in a large number of developmental processes.

Studies of mice and flies like those just mentioned take advantage of the fact that these are premier model systems for the study of development, but are hampered by the difficulty of studying natural populations. In contrast, *Gasterosteus aculeatus*, the three-spine stickleback was chosen for micro-evo-devo studies due to a wealth of recently differentiated natural populations. This fish and its close relatives are found in near-shore marine environments in the Northern Hemisphere. They have repeatedly invaded freshwater habitats from the oceans, particularly as deglaciation proceeded after the last ice age, making every river and stream an independent evolutionary experiment with a geologic time signature. Sticklebacks have consequently been a favorite subject for studies of phenotypic evolution (Bell and Foster 1994), including the genetic basis of developmental changes. For micro-evo-devo, these natural experiments provide many of the advantages of artificial selection experiments, plus the fact that nature has done the work of selection over a much longer time period than is practical in the laboratory. For many traits the resulting differences in phenotypes between founder and derived populations are large enough to make it possible to use the comparative evo-devo toolkit to identify and test hypotheses about the basis of adaptation. This combination of features enables investigators to compare variation in the marine founder population to the products of evolution based on that variation, while investigating the cause of those differences.

For example, many freshwater populations undergo loss of spines and armor that help protect marine fish from predation. In freshwater these features actually increase predation by insect predators, and increase the demand for calcium, which is far more limited in freshwater habitats. In addition to contemporary studies documenting these selective forces, detailed fossil sequences show that the loss of spines and armor takes place gradually over a few 1000 generations (Hunt et al. 2008). Genetic changes that make large contributions to these repeated losses have been traced to cis-regulatory changes at two key developmental genes. The armor plates are greatly reduced by a mutation in the regulatory region of the *ectodysplasin* (*EDA*) gene (Colosimo et al. 2005; O'Brown et al. 2015), which plays an important role in cell-signaling during developmental of the neural crest and ectoderm. Remarkably, the same allele (haplotype) is involved in this adaptation throughout much of the range of the species. It is maintained at low frequency in the marine

population which founds each freshwater population by gene flow from the existing freshwater populations. This enables rapid adaptation upon invasion of a new drainage. In contrast, the genetic basis for loss of pelvic spines in some freshwater populations has been traced to deletions of an enhancer at the *Pituitary homeobox 1* (*Pitx1*) locus (Chan et al. 2010). In this case, the deletion present in each drainage was unique. Several lines of evidence suggest that this enhancer is in a region of the genome with a high mutation rate for deletions. Thus, in both of these cases of armor loss, the supply of the variation is enhanced, potentially explaining why these two regions are responsible for parallel adaptive events.

These dramatic cases of evolution due to fixation of large effect alleles do not explain all aspects of stickleback adaptation. Studies that map the genetic differences underlying many differentiated traits show that such changes typically involve a large number of genetic changes with a range of effects sizes (Peichel and Marques 2017). Even in the case of armor loss, many other genomic regions contribute to the multifarious differences among populations. For example *Eda* has a large effect on plate number, but minor effects on the size of the plates that are produced. A very useful heuristic model for adaptation is that of Fisher's geometric model (Orr 2005) of simultaneous adaptation of many traits. Fisher assumed that each trait has an optimal state at which fitness is maximized, and that the fitness of a phenotype is a smooth function of the distance to that optimum in the space of all possible phenotypes. He also assumed "universal pleiotropy," meaning that each mutation has some effect on all traits, although the combination of effects differs among mutations. When the population is not at the optimum, mutations will increase fitness when they move the phenotypic state closer to the optimum, and otherwise will decrease fitness. When the population is far from the optimum, mutations with large effects can be favored, but as the population approaches the optimum, variants with large effects are no longer favored, even if they would have provided a better initial step towards the optimum. Instead, a series of smaller effect mutations are fixed as the population approaches the optimum. The pattern of effects sizes in the adaptation of stickleback traits matches the expectation under Fisher's geometric model well (Peichel and Marques 2017).

Building Blocks of Micro-Evo-Devo

Many comparisons of evo-devo with traditional micro-evolutionary biology consider the latter to be synonymous with population genetics. The purview of population genetics is generation and inheritance of discrete inherited variants in their genetic context and the study of the forces affecting the fates of those genetic variants within populations. The phenotype plays no necessary role in population genetics once the relative fitness of genotypes is specified, although many specific population genetics models do include a connection between fitness and phenotype, albeit in a usually simplified and rather abstract form. This phenotype-blindness of population genetics has been the subject of scorn not only from practitioners of

evo-devo but also from biologists more concerned with the evolution of phenotypes than genes (Laubichler 2010).

There is, however, another tradition of micro-evolutionary studies based on quantitative traits that is complementary to that of population genetics. The field of quantitative genetics infers the inheritance of phenotypes from phenotypic data on related individuals without directly assuming anything about the underlying genetic variants. The nature of the phenotypic similarities allows the quantitative geneticist to predict the response of phenotypes to natural and artificial selection. Quantitative genetics is a natural way to evaluate important aspects of genetic architecture, such as evolvability (see the chapters ▶ "Evolvability" and ▶ "Variational Approaches to Evolvability: Short- and Long-Term Perspectives"), modularity (see the chapter ▶ "Modularity in Evo-Devo"), and canalization (see the chapter ▶ "Canalization: A Central But Controversial Concept in Evo-Devo"). The generation of new phenotypic variation by mutation or as a plastic response to the environment is also in the purview of quantitative genetics (e.g., Braendle et al. 2010; Houle and Fierst 2013; see also the chapter ▶ "Developmental Plasticity and Evolution").

An important aspect of this quantitative arm of micro-evolutionary biology is that the study of inheritance and selection readily generalizes to the study of complex phenotypes consisting of many potentially interrelated parts (Lande 1979; Lande and Arnold 1983). Almost any morphological phenotype is complex in this sense. Unfortunately, many studies of the evolution of development at all scales make the unrealistic but convenient assumption that phenotypes can be represented by a single measurement or as a set of discrete alternative states. The ability to measure complex phenotypes is increasing rapidly (see the chapter ▶ "Phenotyping in Evo-Devo"), and incorporating these multivariate data is a challenge for all aspects of evo-devo studies (e.g., Pitchers et al. 2019).

Despite capturing largely complementary aspects of evolution, the population genetic and quantitative genetic approaches unfortunately cannot readily be combined into a single comprehensive approach to micro-evolution. They use completely dissimilar formalisms where the parameters in one tradition have no equivalents in the other tradition. For example, population genetics assign fitnesses to discrete genotypes, but discrete genotypes are not identified in the quantitative tradition. The fitness functions estimated in the quantitative tradition cannot be applied to genetic variants that lack phenotype information.

Building GP Maps

Population genetics and quantitative genetics operate on opposite sides of the GP map (Lewontin 1974). It has long been clear that what we need to fuse the complementary advantages of population and quantitative genetics is a GP map. Micro-evo-devo studies necessarily incorporate both population genetic and quantitative genetic concepts, connected through the concept of the GP map (see the chapter ▶ "Genotype-Phenotype Map"). Key advantages of a GP map-based approach include gaining a handle on the empirically challenging concept of

pleiotropy (see the chapter ▶ "Pleiotropy and Its Evolution: Connecting Evo-Devo and Population Genetics"). Without an a priori notion of the map, the only way to study pleiotropy is to estimate effects of genetic variants on the prohibitively large universe of possible traits affected (Pitchers et al. 2019). Furthermore, the GP map concept enables study of how the map itself can evolve (see the chapter on ▶ "Epistasis"), potentially reshaping evolutionarily important parameters, including pleiotropy and evolvability.

The difficulty is that, with a few limited exceptions, GP maps are very poorly known. What knowledge evolutionary biologists have of GP maps usually comes from elsewhere in biology – from mutational screens, and from cell and developmental biology. Often such information initially is no more than a pointer for future work "Look in or around this gene for relevant genetic variation." In addition, there are many vaguer generalizations that we use as proxies for parts of the GP map, such as "premature stop codons have large phenotypic effects" and "synonymous amino acid substitutions will usually have no phenotypic effects."

Traditional developmental and evo-devo studies often build out parts of genotype-phenotype map as a byproduct of researching the function or evolution of genes or pathways. For example, the transcription factor *ovo* in *Drosophila melanogaster* and closely related species (reviewed in Chapter 6 of Wagner 2014) has 3 alternative promoters, 5 different transcripts with different functions and at least 13 enhancers. One transcript is necessary in primordial germ cells, while that and a second transcript act antagonistically to regulate gene expression in the ovary. A third transcript is involved in several aspects of cuticle differentiation, and three enhancers of this transcript generate spatially precise effects on the placement of trichomes (miniature protrusions on the cuticle) that vary among species (McGregor et al. 2007). Thus, work on this one gene has provided us with hypotheses about the function of regulatory and coding regions, some of which are expected to influence many phenotypes, some to have highly targeted phenotypic effects, while some may not have any phenotypic effects. However, note for all this effort, we are far from having any detailed predictions of, for example, what effects that substitution of specific base pairs will have on phenotypes.

A complementary tool that is now being exploited to build GP maps is the genome-wide association study (GWAS). In a GWAS, a set of genotypes are sequenced and their phenotypes are measured. When a statistically robust association between a pair of variants and a phenotype is discovered, we have measured the effect of those variants on a phenotype, one of the crucial parameters of both population and quantitative genetic models (Bastide et al. 2013). Ultimately merging the results of developmental genetic, evo-devo, and GWAS studies coupled with functional verification will allow us to make increasingly detailed GP maps to inform micro-evo-devo studies. An important challenge for GWAS studies is to incorporate pleiotropic effects (Pitchers et al. 2019).

Detailed models of development and the phenotypes it produces is a promising approach to building a hypothesis about GP maps (see the chapters ▶ "Modeling Evolution of Developmental Gene Regulatory Networks" and ▶ "Computational Modeling at the Cell and Tissue Level in Evo-Devo"). For example, a detailed model

of tooth development can reproduce the major features of tooth morphology and provides hypotheses about which aspects of development will respond under simulated selective scenarios (Milocco and Salazar-Ciudad 2020). If such a model were coupled to experimental work in a model organism, this would be a powerful program to build a GP map, and then test its ability to predict micro-evolution.

What Can Micro-Evo-Devo Do for Evo-Devo?

Given the status of micro-evo-devo as a younger step-child of traditional, comparative evo-devo, an important question to ask is what micro-evo-devo can bring to the study of the evolution of development that is distinct from what can be learned using the comparative approach. One entrée to potential advantages of micro studies is to consider the limitations of traditional evo-devo studies.

The first such limitation is what we might call the "discreteness bias" of evo-devo studies. The chosen targets of evo-devo studies are overwhelmingly discrete differences among taxa – the transformation of wings to halteres in insects, the evolution of novel color patterns, the loss of limbs in snakes. The ability to investigate such discrete differences is a strength of evo-devo that only becomes a bias when there is a tendency to equate all interesting developmental changes with the causation of discrete differences. Questions about the evolution of important quantitative characteristics, such as differences in body size and the accompanying allometric changes in virtually every aspect of form are also interesting. Generalizations about the evolution of form are nevertheless being built on the catalog of causes of discrete differences among taxa (e.g., Carroll 2008).

Micro-evo-devo studies generally do not start out to explain discrete phenotypic differences, and thus help balance the scope of evo-devo. For example, Kamberov et al. (2013) studied an amino acid substitution in the human Ectodysplasin A (*Eda*) receptor (*EDAR*). This human polymorphism was targeted for study after population genetic studies revealed that the derived *EDAR* allele has been strongly favored by selection in East Asian and Native American populations over the past 30,000 years. Subsequent studies showed that it was associated with differences in scalp hair thickness and tooth shape in human populations. Kamberov et al. set out to verify that the coding variant was in fact responsible for these phenotypic associations by introducing the amino acid change into the mouse genome. The knock-in mice did, in fact, have 25% more hair follicles whose thickness was enhanced by one cell width, but also revealed 10–25% higher density of eccrine (sweat) glands, increases in branching in the mammary gland by about 25%, and 10%, and lower mammary fat pad size. Subsequent investigations in humans showed that individuals carrying the derived allele also had increased numbers of eccrine glands, as the mouse results predicted. While it remains unclear which of the phenotypic effects of the *EDAR* have been responsible for its spread in this human population, it is a revealing example of positive selection of a highly pleiotropic quantitative genetic variant.

Micro-evo-devo studies are well equipped to study the evolution of continuous traits rather than discrete differences. Patterns in continuous traits can be as relevant

to larger scale evolutionary patterns as discrete differences. For example, there is a long-standing controversy over how much allometry constrains evolutionary patterns. Bolstad et al. (2015) performed artificial selection on the slope of a conserved pattern of allometry for wing shape and size in *Drosophila melanogaster*. In just 26 generations the slopes evolved to be outside the range of slopes in the entire genus *Drosophila*, suggesting that allometry does not constrain wing shape. However, when artificial selection ceased, the slopes evolved back towards the original slope faster than they diverged under artificial selection. They also selected wing shape to evolve without altering allometric slope, but in those lines, there was little evidence of natural selection for the ancestral state. Consequently, Bolstad et al. interpreted this counter-selection as the result of a pleiotropic burden (see the chapter ▶ "Concept of Burden in Evo-Devo") on allometry, rather than selection on wing shape per se. This suggests that this allometric relationship is constrained by pleiotropy with unknown aspects of development, not by a lack of genetic variation in slope.

It is more challenging to use the micro-evo-devo toolkit to study the opportunity for discrete changes in morphology, as, in many cases, discrete phenotypic variation is not present within populations. Studies of mutational effects can sometimes reveal discrete differences, such as the transformations of cell fate in the vulva of *Caenorhabditis elegans* (Braendle et al. 2010; see the chapter ▶ "Devo-Evo of Cell Types"). In natural isolates, the pattern of cells is nearly invariant, but after mutations are allowed to accumulate for many generations, this pattern becomes more variable. Mutations in different genotypes generated different rates of transformations, and there were hints that the sorts of transformations that occur at the highest rate are more typical of those found in related species. In rare instances, populations contain individuals of two or more discrete morphological types, and these can be used to study the underlying developmental basis.

The second potential limitation is "endpoint bias." Having demonstrated the role of a particular gene in shaping phenotypic differences between taxa, it is natural to equate the evolution of the feature in question to the evolution of that gene. For example, Kopp et al. (2000) demonstrated that the gene *bric a brac* (*bab*) has evolved a novel, sex-specific regulatory role in the last two abdominal segments in some *Drosophila* species. This novel regulation enables male flies to evolve pigmentation and shape differences from female flies in those segments. Having demonstrated this, Kopp et al. then proposed a parsimonious selective scenario whereby selection on *bab* expression by sexual selection is responsible for the evolution of the novel male-specific features. However, an alternative hypothesis is that the initial evolution of sexual differentiation took place by some other mechanism, and the recruitment of *bab* into its current regulatory role took place later.

We do not criticize Kopp et al. for putting forward their parsimonious hypothesis, but there are theoretical and empirical grounds to suspect that the differences in development between species may be different from the mechanisms by which the phenotypes diverged in the first place. On the empirical side, developmental systems drift (DSD) is a well-known and common phenomenon whereby the developmental basis of traits diverges even though the trait has remained roughly constant since

divergence from the common ancestor. One potential explanation for this phenomenon is literally genetic drift – that there are completely equivalent ways to make the same phenotype, and this allows fixation of alleles that transition the system from one developmental solution to another (see the chapter ► "Developmental Systems Drift"). Perhaps a more likely explanation for DSD is suggested by Fisher's geometric model (Orr 2005). When the population is far from the optimum, the variants with large effects initially fixed are likely to move some traits farther from their optimal state. Once such an imperfect mutation becomes fixed in the population, mutations fixed at later steps in the adaptive process will rectify the pleiotropic side effects of those early steps. The model predicts that adaptation will tend to zig-zag towards its final state, rather than proceeding by a single fixation event that achieves the optimal state. Thus, the developmental solution to an adaptive challenge may well shift once the population is near the optimum state. For example, a plausible scenario is that the *bab* enhancer now regulating coloration in male abdomens was recruited to correct the side effects of some earlier mechanism of achieving a similar phenotype. Now consider a second adaptive event that primarily involves different traits. The pleiotropic side-effects of that event may perturb development of our focal traits, again engendering evolution to restore that optimum phenotype (Pavlicev and Wagner 2012).

How these inferred shifts in the developmental basis of evolved states occur has not yet been addressed by micro-level experiments. Artificial selection and experimental evolution studies of developmentally interesting organisms have generated very few allelic fixation events due to their short duration, precluding examination of this issue. Natural experiments such as the repeated invasions of sticklebacks into freshwater habitats have a greater range of time depths and offer the opportunity to evaluate this possibility, although we know of no attempts to do so.

Do Micro Events Affect Long-Term Evolution?

There are many reasons to suspect that the micro-evolutionary events and processes that we can observe directly are not relevant to evolution above the species level and over long time periods (see the chapter ► "Macroevolution"). For example, if the cis-regulatory changes of large effect in fact dominate long-term evolution (Carroll 2008), it is possible that the vast majority of the abundant polygenic variation within contemporary populations could have no long-term importance. Some paleontologists have long argued for such a discontinuity between micro-evolutionary processes and macro-evolutionary patterns on other grounds (Gould 2002). Even quantitative geneticists have generally doubted that the parameters measured within contemporary populations are stable enough to predict evolution over long time periods. Indeed a study that integrates data on evolutionary rates of body size evolution across time scales from generations to hundreds of millions of years suggests a discontinuity reminiscent of punctuated equilibrium (Uyeda et al. 2011). Up to a time depth of about one million years, body size of descendant populations or species always remains within a factor of about 1.5 of the ancestral

value, but at longer time scales larger changes in body size become increasingly likely. It is not clear what processes can explain this apparent discontinuity, but it certainly suggests that it is very unlikely that one of these rare large changes will be encountered in any particular population at the present. These doubts about relevance are likely an important factor in the relative paucity of micro-evo-devo studies.

The result, however, is that we have relatively little data we can use to empirically test whether micro- and macro-evolution are in fact related to each other. Recent studies suggest that in some taxa there may be a strong connection between micro processes and macro patterns, despite the many reasons there should not be. Perhaps the most striking example is the recent comparison of the diversification of wing size and shape in the dipteran family Drosophilidae (Houle et al. 2017). This contribution estimated the phenotypic variation produced by mutation in 21 aspects of wing size and shape (Houle and Fierst 2013) in *Drosophila melanogaster* and compared that variation to standing variation in the same species and to variation among over 100 Drosophilid species that diversified over at least 30 million years. Both mutational and standing variation within *D. melanogaster* were highly correlated with the rate of evolution in the family. Wing shape evolves slowly in this group, so it is possible that this strong relationship is a peculiarity of a suite of slow-evolving traits. However there are hints of similar patterns in traits that show higher rates of evolution as well, such as the blossoms of the plant genus *Dalechampia* (Bolstad et al. 2014). The relevance of micro-evo-devo studies to the broader field of evo-devo should not be lightly dismissed.

Cross-References

- ▶ A Process-Based Approach to the Study of Flower Morphological Variation
- ▶ Computational Modeling at the Cell and Tissue Level in Evo-Devo
- ▶ Concept of Burden in Evo-Devo
- ▶ Developmental Evolutionary Biology (Devo-Evo)
- ▶ Developmental Plasticity and Evolution
- ▶ Developmental Systems Drift
- ▶ Eco-Evo-Devo
- ▶ Epistasis
- ▶ Evo-Devo's Contributions to the Extended Evolutionary Synthesis
- ▶ Evolvability
- ▶ Genotype-Phenotype Map
- ▶ Macroevolution
- ▶ Modeling Evolution of Developmental Gene Regulatory Networks
- ▶ Modularity in Evo-Devo
- ▶ Phenotyping in Evo-Devo
- ▶ Variational Approaches to Evolvability: Short- and Long-Term Perspectives

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