4 The Shape of Things to Come: *Evo Devo* Perspectives on Causes and Consequences in Evolution

Armin P. Moczek

Introduction

When I was a young and innocent postdoctoral researcher hunting for the elusive tenure track position, I would begin my seminars by briefly highlighting the major contributions made by alternative frameworks in conceptualizing what matters in directing evolution. Showing a slide of Darwin's finches, I would emphasize the role of adaptive evolution and the view of organisms as Swiss army knives—accumulations of gadgets, each with a specific function honed over time. Showing a drawing of a prehistoric small mammal gnawing on a dinosaur carcass, I would highlight the role of chance and accidents in the diversification of life on earth. And lastly I would show a drawing of a bird embryo squeezed to its limits within an egg and emphasize the role of developmental constraints in determining where and where not evolution may be allowed to go. I would close this introduction with three major conclusions: First, all three of these perspectives have been incredibly illuminating. Second, they are not mutually exclusive. Third, they are all roughly equally useless when it comes to understanding the origins of novelty in evolution because selection cannot select for traits that do not yet exist, accidents can only sort among preexisting variation, and constraints only limit options, but by themselves do not create new ones (Moczek 2008). Instead I would posit that how the origin of novelty can be integrated within a framework of descent with modification, how novel complex traits may originate from within the confines of ancestral variation, the baby steps of innovation needed to eventually yield the first limb, wing, eye, feather, photic organ, and so on all remain remarkably poorly understood in spite of over 150 years of vibrant evolutionary biology since the publication of the *Origin of Species* (Darwin 1859). And then I would say that my research program addresses these shortcomings by integrating the role of development into our understanding of speed and direction in organismal evolution, in particular in the origins of novelty, and that I will finally resolve this long-standing, foundational challenge to evolutionary biology. And that you really should strongly consider hiring me.

About 15 years have passed since and it is appropriate that I look back and assess where we stand—the larger field of evolutionary developmental biology (*evo devo*) in general, and my own research program as one of its many representatives—with respect to our abilities to contribute meaningfully to our current understanding of the evolutionary process, in particular with respect to challenges for which previous approaches and schools of thought have struggled to find resolution, such as innovation and the origins of novelty. Specifically, in this chapter I will begin by reviewing a few key terms and concepts, and the creative tensions between them, that will be critical for subsequent discussions. Secondly, drawing from the work of others as well as my own, I will then highlight examples that illustrate how, on what levels of biological organization, and on what level of causation, an understanding of how organisms build themselves enriches our understanding of how and why they evolve the way they do. Lastly, I will discuss some of the challenges that remain, and in particular conceptual challenges *evo devo* is now itself encountering, and opportunities for their resolution. Let us set the stage, however, with a brief discussion of some of the conceptual constellations that led to the birth of *evo devo* as a discipline, and which motivate many of its practitioners, including myself.

What Can an Evolutionary Biologist Possibly Learn from Studying Development?

This was the question I would hear during every single meeting I had with my dissertation committee while in graduate school. It was always posed by the same faculty member who shall remain unnamed, a highly accomplished evolutionary biologist and population geneticist, who to this day I respect very much, and who posed his question not to tease me: instead, he truly did not understand why anyone interested in understanding evolution's paths would bother learning about how development works. In this of course he was and is not alone—it is a mindset that scientists like myself encounter to this day. *Evo devo*'s contributions to our understanding of the evolutionary process are often either considered modest at best, or alternatively, thought of as not really evolutionary in nature. So how did we get to this point?

Evolutionary biology as a discipline first emerged in the first half of the twentieth century by integrating natural selection and Mendelian inheritance into the then-coalescing framework of population genetics (Mayr 1982). In the decades that followed, evolutionary biology continued to expand and mature into a highly sophisticated and successful framework able to address a broad range of biological phenomena. In the process, several key concepts and dichotomies became deeply engrained in how we conceptualize organismal evolution in research and how it is taught in our courses: most importantly, we grew to understand phenotypes as rooted in genes and genomes, and as long as phenotypic variation could be associated with genetic variation in some way, this enabled the opportunity to conceptualize, and thereby equate, *phenotypic* evolution as a change in the *genotypic* composition of a population over time (Laland et al. 2015). Doing so removed the need

to understand exactly how genotypic information and variation manifested in phenotypes and phenotypic variation. As a by-product, this then removed any need to understand how organisms are built during development from understanding how they evolve over time. The resulting quantitative framework enabled important advances in our understanding of the nature of diverse evolutionary phenomena, though a subset of challenges stubbornly resisted resolution, including as already noted the questions surrounding the origins of novel complex traits and the corresponding major transitions and radiations they enabled. Given their entrenchment in deep time, and the lack of phenotypic variation accessible to quantitative and population-genetic approaches, standard evolutionary biology struggled to generate satisfactory answers and had stopped trying by the time I had entered graduate school. The resulting disconnect between what I considered to be among the most important questions in evolution, and the tools I was being taught in my upper-level graduate evolution classes, caused me to look elsewhere, and the then rapidly transforming field of *evo devo* very quickly revealed to me novel ways to both conceptualize, and empirically interrogate, the nature of innovation in evolution.

What Is an Evolutionary Novelty?

Prior to the advent of *evo devo*, Ernst Mayr (1960) defined novelty as "any newly acquired structure or property that permits the assumption of a new function," which parallels corresponding statements as far back as Lamarck and Darwin, holds intuitive appeal, yet runs into trouble when we try to use it to derive hypotheses regarding how novelties might originate: selection can only act on traits that already exist, but if they already exist in some shape or form they are no longer exactly novel. Something else is needed to account for the initiation of novelty independent of future functionality. A second definition was proposed by Müller (1990), who defined novelty as "a qualitatively new structure with a discontinuous origin, marking a relatively abrupt deviation from the ancestral condition." This definition remained neutral regarding functionality, but left it up for interpretation where quantitative variation ends and qualitative distinctness begins. How different is novel? This is where a third definition, proposed by Müller and Wagner (1991), stepped in to provide what seemed like an iron-clad cutoff: "A morphological novelty is a structure that is neither homologous to any structure in the ancestral species or homonomous to any other structure in the same organism." Now novelty began where homology ended. But where does homology end? Traditionally, and as taught in the introductory zoology classes I took in Germany in the early 1990s before moving to the United States, classic homology criteria included relative position, intermediate forms, and the all-encompassing special qualities, and they neatly dichotomize traits into those that are homologous and those that are not (Remane 1952). *Evo devo* very swiftly forced a revision of this framework into a far more complex, nuanced, and layered understanding of homology, for two major reasons. First, it forever rejected the notion that the extraordinary phenotypic diversity that exists on the level of organisms must somehow be paralleled by a corresponding diversity

in genetic and developmental mechanisms. Instead, researchers now recognize that the developmental genetic underpinnings of phenotypic diversity are remarkably conserved, and that highly divergent organisms rely on much of the same developmental mechanisms to instruct the building of very different, and by conventional criteria clearly non-homologous, organs and structures (reviewed in, for example, Shubin, Tabin, and Carroll 2009; Held 2017; and see below for concrete examples). And we had to recognize the existence of the *opposite* constellation as well: traits that by conventional criteria are clearly and unambiguously homologous may form during development in surprisingly different, *non-homologous* ways, a phenomenon we now recognize as developmental systems drift or phenogenetic drift (Weiss and Fullerton 2000; True and Haag 2001). *Evo devo* thus forced a transformation of our understanding of homology away from a neat and discrete black and white to layered set of shades of gray (Wagner 2014). At the same time, as we will see next, it brought us significantly closer to understanding the origins of novelty in ways no previous discipline had been able to achieve.

Novelty and Diversity from the Confines of Ancestral Variation

(a) Cooption, Parallelism, and the Modular Nature of Development

By discovering the remarkable conservation of developmental building blocks and processes that characterizes phenotypic diversity, *evo devo* forced a view of organismal diversity akin to that of Lego creations: rather than being shaped primarily or solely by adaptive responses to selection pressures, diverse organisms emerged as the modified re-assemblages of the same and seemingly very limited pool of genes, developmental pathways, and morphogenetic processes. Clearly, natural selection remained a leading force in the creation of organism–environment fit, but one that suddenly had to draw from a heavily restricted pool of resources from which to generate diversity. No surprise that many embraced the new *evo devo* findings pouring in during the 1990s as a reflection of overwhelming evidence for *developmental constraint* on what evolution might otherwise be able to accomplish. If diversification seemed heavily constrained, then how anything *novel* could ever emerge in the process was anyone's guess. While I sense that many evolutionary biologists looking in on the discipline of *evo devo* have retained the perspective to this day that the best understanding development can do for an evolutionary biologist is to understand *the limits it imposes on diversification*, *evo devo* itself managed to move on, in large part because of the realization that what may act as a constraint in one context may provide critical opportunities in others, and that diversity and novelty may have evolved not *in spite* of the deep homology of genes, pathways, and processes across phyla, but *because of it*. Two examples will help to illuminate this perspective.

The first concerns the eyes of vertebrates, insects, mollusks, or jellyfish—morphologically distinct structures that arise in disparate embryological contexts (reviewed in Shubin et al.

2009). Eyes are thus assumed to have originated independently many times in different phyla, and traditionally they have been interpreted as representing remarkable cases of convergent evolution. Thanks to comparative developmental studies, however, we now know that even across phyla homologous transcription factors help specify and organize eye formation, while homologous opsin proteins provide the light-absorbing properties needed to convert incoming photons into outgoing action potentials (Oakley 2003). Further, the interneurons involved in processing this visual input—that is, the optic lobe of *Drosophila* and the retina of vertebrates—while only analogous in function, are both specified by homologous transcription factors, both project to visual centers of their respective brains, and both subsequently express yet again homologous transcription factors to aid in further integration of signal processing (Erclik et al. 2008; Shubin et al. 2009). Collectively, these data suggest that rather than being simply the product of convergent evolution, eye diversity across phyla may more appropriately be understood as reflecting parallelisms, enabling the evolution of lineage-specific eyes by utilizing the same ancestral toolbox of patterning mechanisms, visual pigments, cell types, and cellular circuitry.

The second example concerns appendage formation. While the legs of flies and mice, the tube feed of echinoderms, the siphons of ascidians, or the horns of scarab beetles have very little in common in terms of strict homology, they all share that they are outgrowths whose development requires some kind of mechanisms specifying which cells will adopt a distal fate, and which will be proximal, anchoring the outgrowth in the remainder of the body. We now know that to do so, all of them, despite the enormity of phylogenetic distance among them, share the use of the same genetic regulatory cascade including the use of a key transcription factor (e.g., *Distal-less* in insects, *Dlx* in vertebrates) and shared downstream effector genes, a machinery likely already in existence in the earliest bilaterians (Panganiban et al. 1997; Mercader et al. 1999; Moczek et al. 2006; Moczek and Rose 2009). As before, parts of an ancient developmental toolbox became reused over and over again, independently in very different lineages, in this case to enable the formation of various kinds of outgrowths, limbs, and appendages.

Collectively, these and by now hundreds of similar examples illustrate, on one side, the modular nature of developmental processes: a module exists that specifies proximo-distal polarity and will do so regardless of exact context, just like a module exists that can pattern the formation of cell types that can be assembled into light sensors. Secondly, what may have originally been perceived as a constraint, because it seemed there is developmentally only one way to make an eye or a limb, may now be simultaneously recognized as a set of preexisting opportunities in disguise. Put another way, while there may indeed only be one way to specify proximo-distal identity in development, if a lineage ever needed to add that ability to whatever novel context, it was already endowed with a preexisting developmental toolbox ready to do just that. If light perception offered new ecological opportunities, the developmental genetic properties necessary for the specification of some type of light-sensing organ did not need to be evolved *de novo*, instead it already lay in wait

in every bilaterian lineage. This creative ability of development to focus evolution along specific productive directions becomes further apparent if we consider the major avenues along which modest developmental modifications enable the modular nature of development to yield remarkable phenotypic diversity and novelty, as discussed next.

(b) Facilitating Developmental Evolution along Four Dimensions: The Power of "the Four Hs"

The modular nature of development allows developmental units, from genetic pathways to morphogenetic processes, to be executed semi-independently from other modules. The four Hs—heterotopy, heterochrony, heterometry, and heterocyberny—all illustrate that no new modules are needed to generate profound diversity via relatively simple changes in the execution of modules relative to each other.

(i) Heterotopy refers to an evolutionary change in the precise location of a developmental event. No *new* modules are needed; instead all that is altered is the ontogenetic location of their actions relative to each other. Executing "old" modules in novel spatial contexts is now recognized as yielding some of the most spectacular examples of innovation in evolution: For example, the recruitment of appendage patterning genes ancestrally used to establish the proximodistal axis of arthropod legs, antennae, and mouthparts into the dorsal head and pronotum of beetles represented key contributions to the origin and subsequent rapid diversification of beetle horns now used in male combat (Moczek et al. 2006; Moczek and Rose 2009). Recruitment of some of the same genes onto the wing surfaces of butterflies facilitated the developmental evolution of wing spots, now used in mate choice and predator avoidance (Brakefield et al. 1996). Heterotopy can change—not just add parts to where they did not exist before; it can also contribute old functions to newly positioned traits: for example, cooption of signaling via the *hedgehog* and *doublesex* pathways now allows beetle horns to adjust their development in a highly nutrition responsive manner, yielding alternative male morphologies with species as a consequence (reviewed in Moczek and Kijimoto 2014; Casasa, Schwab, and Moczek 2017). In all of these cases the Lego analogy of organismal development and evolution is perhaps most apt and obvious: novel traits and functions may arise with ease by placing old building blocks in new places. However, additional routes to novelty exist, perhaps more subtle in nature, but potentially just as consequential.

(ii) Heterochrony refers to a change in the timing of developmental events relative to each other from one generation to the next. Heterochrony can occur on any level of biological organization, from gene regulation to behavior. All heterochronies share, however, that, as with heterotopy, no new events are introduced; instead, all that is altered is the ontogenetic timing of their actions relative to each other. The consequence of heterochrony range from subtle and quantitative to profound and qualitative. For example, bats and kiwis arrive at relatively large and smaller forelimbs than other quadrupeds by advancing and delaying the onset of forelimb growth, respectively (Richardson 1999). The forelimbs of

marsupials also develop earlier compared to placental mammals, enabling the marsupial embryo to climb into the pouch and suckle (Smith 2003; Sears 2004). Heterochrony can change not just the relative sizes of parts but also their number: for example, in the lizard genus *Hemiergis*, the duration of *sonic hedgehog (shh)* activation within the limb bud determines whether the bud gives rise to 3, 4, or 5 digits (Shapiro, Hanken, and Rosenthal 2003).

Heterochrony's consequences, however, can also be truly profound: A dramatic example is the origin of holometabolous development in insects, as seen in butterflies, moths, beetles, or bees, which develop from an embryo to an immature larva, which undergoes several larval-to-larval molts before molting into a pupa, and ultimately an adult. The pupal stage thus effectively decouples the larval from the adult stage and is credited with having allowed larval holometabolous insects to utilize feeding niches otherwise unavailable to other insects, culminating in the dramatic divergence in form, physiology, and behavior between larval and adult stages of extant holometabolous insects. This is in contrast to hemimetabolous development as observed in, for example, grasshoppers or cockroaches, in which embryos molt via a brief pronymph stage into a nymphal stage, which in many ways resembles a miniature and incomplete version of the final adult. Through a series of nymphal-to-nymphal molts animals grow in size, culminating in a final nymphal-to-adult molt (Gullan and Cranston 2014). The most widely held hypothesis regarding the origin of holometabolous development postulates that holometabolous larvae are homologous to hemimetabolous nymphs, and that the origin of holometabolous metamorphosis was made possible through the invention of the pupal stage which, consequently, lacks a homologous counterpart among the Hemimetabola. Recent work challenges this long-standing view and argues that the holometabolous pupa instead arose from a *compacting* of the nymphal stages into a single life stage, thus making pupal and nymphal stages homologous (Truman and Riddiford 1999). The holometabolous larvae in turn arose as an *elaboration* of the hemimetabolous pronymph stage. The pronymphal stage of hemimetabolous insects is a distinct stage directly following the embryo, but it is so brief and ephemeral that it is spent entirely to largely while the animal is still inside the egg. Compelling evidence now exists that supports the hypothesis that the holometabolous larva may indeed have arisen through a "de-embryonization" of the pronymph stage, converting a largely embryonic stage into a free-living larva. The hemimetabolous nymphal stages, in turn, collapsed into what we now recognize as the holometabolous pupa. Consequently, a three-part life cycle already existed prior to the origin of holometabolous development, which instead arose via heterochronic changes in the endocrine regulation of growth and molting. If correct, no origins of a new stage are needed, instead heterochronic modifications of preexisting stages may have sufficed to fuel the single-most important developmental transition in insect evolution.

(iii) Heterometry is commonly defined as an evolutionary change in the amount of a gene product but could also be applied to higher order developmental products, such as hormone titers. Like heterochrony, it seems like a subtle change which in turn should only

have subtle consequences. One of the best examples illustrating the opposite is the developmental evolution of Darwin's finches, via heterometric changes in two genes, bone morphogenetic protein 4 (Bmp4) and calmodulin (cal), which both encode proteins that through different routes promote cell division and thus tissue growth (Abzhanov et al. 2004, 2006). Experimental and modeling work shows that evolved changes in the expression levels of both genes are sufficient to explain the diversity of beak shapes among Darwin's finches, and that experimental induction of a subset of these changes in chick embryos results in matching changes in beak formation (Wu et al. 2004, 2006).

(iv) Heterocyberny, lastly, is a term few seem to use, but it nevertheless illustrates an important concept: an evolutionary change in *governance*, that is a change in the upstream regulation of a conserved downstream process (Gilbert and Epel 2009). Used most broadly, it refers to the process whereby initially environmentally induced traits may over generations become genetically stabilized and incorporated into lineage's norm of reaction. We will return to this broad notion of heterocyberny toward the end of this chapter; for now I want to emphasize that evolutionary changes in upstream regulation can of course also occur on many other levels. As before, no new modules or building blocks need to be introduced, instead both up and down-stream components already exist; all that changes is the nature of interaction between them. Evolutionary developmental geneticists now broadly recognize, for example, the ease with which transcription factors acquire novel targets, even in traits that themselves constitute relatively recent evolutionary inventions: for example, the somatic sex-determination gene *doublesex (dsx)* regulates the relative size and sex-specific expression of evolutionary novel beetle horns, just like it regulates the same features of much more ancient traits, such as genitalia. Yet in horns it acts on a largely non-overlapping repertoire of target genes (compared to genitalia or brains), suggesting that both heterotopic recruitment of novel regulators (such as dsx) and novel target genes into their governance can occur with surprising ease (Ledon-Rettig, Zattara, and Moczek 2017).

In summary, heterotopy, heterochrony, heterometry, and heterocyberny all illustrate that much diversification and innovation may be possible without the need to generate new genes, pathways, or cell fates. Instead, phenotypic diversity emerges through heritable changes channeled along four simple developmental axes—developmental time, developmental location, quantity of developmental product, and nature of regulatory interactions. Lastly, combinations of these four processes operating sequentially or at the same time have the power to further potentiate the developmental degrees of freedom available for rapid evolutionary diversification and innovation.

One may ask then which mechanisms in turn enable developmental processes to be so modular in developmental time, space, upstream regulation and downstream output? The reasons for this derive to a significant degree from the fact that the mechanisms in question are themselves highly modular, and on a variety of levels (reviewed in Carroll, Grenier, and Weatherbee 2004; Gerhart and Kirschner 2010; Gilbert 2013): for example, cellular transduction pathways convert signals external to a cell, such as information on nutrient

availability or position, into signals that enter the nucleus and affect gene expression. Cellular transduction of information primarily takes the form of on/off switches, which are in operation all the time during the life of a cell, in all cells and tissues, developmental stages, and in response to a remarkable diversity of external cues, soliciting a corresponding diversity of intracellular responses. Collectively, this diversity of regulatory decisions reliant upon signal transduction pathways is mindboggling, especially when juxtaposed to the comparatively minute number of transduction pathways that facilitate it. All of those share that they are ancient, predating the traits or processes they regulate in extant organisms by at times billions of years. They also share remarkable degrees of conservation across phyla, and an exquisitely fine-tuned, robust, and reliable nature of interactions among their respective component parts. And lastly, they all share that their modular and combinatorial re-use across diverse contexts facilitates precise developmental decision-making, yet without having to evolve a comparable diversity of switch mechanisms (Gerhart and Kirschner 2010). Transcription factors by themselves, too, contribute modularity, most strikingly through their highly combinatorial action in regulating gene expression. Precise combinations of transcription factors are needed to drive gene expression in specific contexts, and subtle changes in the timing or location of a single transcription factor may suffice to generate heterochronic or -topic developmental changes, without resulting in negative developmental consequences in other aspects of phenotype formation elsewhere, and without the need to evolve new factors for new developmental decisions (Carroll et al. 2004). Lastly, cis-regulatory elements, or CREs, are those genomic regions transcription factors bind to regulate gene expression. CREs are themselves highly modular, with different CREs enabling different facets of a given gene's expression, such as expression in specific locations, developmental stages, cell types, and so on. The highly modular nature of CREs then allows each facet to be regulated—and to evolve—semi-independently, again minimizing pleiotropic constraints (Prud'homme, Gompel, and Carroll 2007). As before, by relying on a preexisting and clearly finite arsenal of building blocks, in this case signal transduction pathways, other transcriptional regulators, and the respective DNA binding sites they interact with, developmental systems are able to generate diverse and novel regulatory settings without having to generate novel regulatory machineries.

On different levels of biological organization we thus see how the nature of developmental processes establishes the degrees of freedom along which development evolution may proceed more easily than others, putting us in a position to understand not just the developmental basis of evolutionary changes but also the creative potential development possesses to facilitate diversity, and doing so not despite the conservation of its building blocks, but because of it. In our quest to understand why evolution unfolds the way it does and not some other way, a comparative developmental perspective thus makes a unique contribution, one no other framework can provide. I would like to close this section by highlighting two concrete case studies to fully illustrate the power and promise of this approach.

Creating a Landscape for Channeling Innovation and Diversification—Two Case Studies

(a) The Evolution of *Drosophila* Wing Pigmentation Patterns

In an important series of studies, Prud'homme and colleagues (reviewed in Prud'homme et al. 2007; see also Wittkopp, Carroll, and Kopp 2003; Gompel et al. 2005; Prud'homme et al. 2006) explored how the developmental architecture that governs the patterning of the fly wing has channeled the subsequent evolution of wing pigmentation patterns. A large body of work has documented the patterning mechanisms that help establish different aspects of the *Drosophila* wing, such as compartmentalization into an anterior and posterior portion, the identity and location of veins, patterning of the distal margin, and so on. All of this is accomplished through transcription factors whose localized expression and action in conjunction with cofactors impart location-specific developmental fates. Collectively, this establishes what is often referred to as the transregulatory landscape of the wing, in existence at a time when most of the wing has grown, and in particular, when in a subset of cycclorrhaphan flies (to which *Drosophila* belongs) pigment synthesis begins and ultimately results in specific, highly diversified wing patterns.

Wing pigmentation patterns result from the conversion of precursor metabolites into visible pigments via the action of specific enzymes operating in a locally restricted manner along the two-dimensional surface of the wings, and as such constitute a developmentally and experimentally very tractable trait. Prud'homme and colleagues were able to show that even though pigment synthesis is a developmental process completely independent of that of wing formation and patterning, the evolution of *pigmentation patterns* on the wing nevertheless integrated both processes in a way that informs our understanding of how the nature of developmental processes both facilitates and biases evolutionary routes to novelty (Wittkopp et al. 2003; Gompel et al. 2005; Prud'homme et al. 2006). Specifically, this work showed that redeployment of preexisting transcription factors and their location-specific expression on the wing into the regulation of pigment-production facilitated the location-specific expression of pigmentation patterns. Recruitment of *different combinations of transcription factors* in different lineages then facilitated the corresponding lineage-specific divergence of pigmentation patterns, but one that was at least in part predictable by the transregulatory landscape already in existence on the wing prior to origins of the first pigment spot.

This work led Wagner and Lynch (2008) to postulate the *Christmas tree model of morphological evolution*, which equates the tree's branches as a reflection of the (preexisting) regulatory landscape of a trait or organism, characterized by the spatio-temporal distribution of transcription factor expression domains. Novel traits, or ornaments in the context of the model, can then be added most easily to existing branches. Or put into fly wing context: pigmentation pattern diversity is preconfigured by the combinatorial possibilities of this regulatory landscape (Wagner and Lynch 2008). This model explains not just the

patterns that were able to evolve in different lineages but also the many examples of parallel pattern evolution, the ease with which some patterns can be lost and (re)gained, and so on. More generally it paints a picture of developmental evolution that converts a constraint into scaffolding for novel diversity, a notion we have by now encountered on several levels in this chapter. At the same time, it assumes a specific polarity: transcription factors create a regulatory landscape, and subsequent innovation and diversification are then shaped by this landscape. Or: branches specify locations for new ornaments, rather than the other way around. Work since suggests, however, that innovation itself, once successful, may well create novel regulatory landscapes, or in the language of the Christmas tree model, that the branches of the tree do not just provide opportunities for new ornaments but respond to new ornaments by growing into previously unoccupied space. Our last example seeks to illuminate this perspective.

(b) The Regulatory Landscape Is Not Static: Innovation on the Dorsal Head of Insects

From the stalks of stalk-eyed flies and the weevil rostrum to the cephalic horns of dung beetles, the dorsal head of adult insects has emerged as an evolutionary hotspot for innovation and diversification (Grimaldi and Engel 2005). At the same time, the insect head has been in existence for at least 420 million years, and the developmental genetic network that patterns head formation is even older, manifest in a remarkable degree of conservation across phyla. Recent work on the horned beetle genus *Onthophagus* has documented that the first position for head horn formation that evolved, and the one now most commonly observed in extant taxa, coincides with the boundary between the clypeolabral and ocular head segments (Busey, Zattara, and Moczek 2016). These segments are first specified during embryonic development, but the mechanisms that specify the boundary between them appear to have been repurposed much later in late larval and pupal development to provide positional information for where horns are to be integrated within the future adult head. Other horn positions also evolved, but did so much more recently and are thus found in far fewer extant species. Up to this point this narrative matches what we have seen thus far—a preexisting regulatory landscape channels novelty—in this case horns used as weapons in male combat—down specific evolutionary avenues. But subsequent studies paint a more complex picture.

Studies on embryonic head development in *Tribolium* beetles implicate the interplay between two transcription factors, *six3*/*optix* and *orthodenticle (otd)* in establishing the clypeolabral-ocular segment boundary (Posnien et al. 2010, 2011). Both genes play critical roles in embryonic head formation in all bilaterians studied, yet their roles in postembryonic development (e.g., during larval, pupal, and adult development) are much less well known. One major exception constitutes *otd*, which in *Drosophila* plays a critical role in late development through promoting the development of ocelli, three single-lens eyes positioned along the posterior midline of the dorsal adult head (Blanco et al. 2009). Following *otd*-inactivation, these ocelli no longer form. Most insect orders possess ocelli,

though it is presently not known whether ocellar development is also under the control of *otd* in these other orders. What is known, however, is that almost all beetles have secondarily *lost* ocelli. Further, experimental down-regulation of *otd*, while lethal in *Tribolium* embryos, has no phenotypic consequences during the formation of the adult dorsal head of the same species (Zattara et al. 2016). Even though *otd* is expressed during adult head formation in *Tribolium*, this expression appears functionless. So far so good.

However, similar experiments in the horned scarab beetle genus *Onthophagus* yielded completely different outcomes. Here, *otd* emerged as absolutely critical for the proper patterning of the dorsal head, including the positioning of cephalic horns. Further, while its function in embryonic development is intimately tied to that of *six3*, that interdependence no longer exists at later developmental stages. It is tempting to speculate that *otd* may have been freed up to evolve this novel function because of the putative secondary loss of its role in regulating ocelli formation in the same part of the dorsal head, prior to the evolution of the first horns. Alternatively, *otd* expression in adult beetle heads may simply be an embryonic leftover, which *Onthophagus* capitalized upon and recruited into the context of horn formation. Regardless of how *otd* arrived at its novel role in *Onthophagus* development, the most important finding, however, is that things did not stop there. Instead, *otd*-specified horn-bearing head regions acquired all sorts of additional functions via the recruitment of a secondary set of pathways: for example, recruitment of the somatic sex-determination pathway enabled horns to be expressed solely in males and exaggerated under high nutrition, facilitating the evolution of both sexual dimorphisms and highly positive allometries in males only (Kijimoto, Moczek, and Andrews 2012). Further recruitment of the hedgehog-signaling pathway enabled the evolution of a complementary function—active suppression of horns under low nutrition only—enabling the evolution of alternative-horned and hornless male phenotypes cued by nutritional conditions experiences as larvae (Kijimoto and Moczek 2016). Additional pathways include signaling via the insulin and serotonin pathways, again pathways that as best as we know play no role in dorsal epidermal head development in insects, yet appear to have been recruited into this context once the opportunity to operate on a new module—horn-forming head regions—existed (Casasa et al. 2017). More generally, these observations suggest that the Christmas tree model of morphological evolution may need to be replaced perhaps by a Romanesco broccoli model, where each addition of a new ornament begets the fractal-like addition of a new branch, a new whirl, offering yet more opportunities for subsequent ornamentation.

What *Causes* Does *Evo Devo* Contribute?

This chapter was meant to explore the contributions made by *evo devo* to our understanding for why and how evolution unfolds the way it does. It is easy to get lost in the details and idiosyncrasies of the many case studies of developmental evolution, and

thus worth to step back and ask: what *causes* in evolution does *evo devo* contribute that may not have been considered prior to the existence of the field? What here is truly new?

For starters, *evo devo* more than any other discipline offers a mechanistically concrete understanding of how traits come into being, and how the underlying processes have to be modified to yield novel trait variants. It is one thing to associate trait variation with allelic variation in a population or to map quantitative trait loci to disease phenotypes. It is a much deeper explanation to also understand how some genetic variants but not others result, for instance, in altered DNA binding of proteins, the circumstances under which this may facilitate the expression of old gene products in new locations or developmental time points, yield the corresponding induction of cellular differentiation events and respective organ formation, and so on. As such *evo devo* offers richer, perhaps more satisfying explanations of what mechanistic causes underlie evolutionary changes. But does it also offer qualitatively novel causes previously unconsidered?

I would posit that it does, on at least two levels. First, *evo devo* offers what has been called a lineage explanation (Calcott 2009) for biological diversity, including novelty. In other words, it offers the opportunity to understand developmental evolution and innovation as a sequence of events, where one event is needed to enable another to take place, leading eventually to a final outcome. Because the traits of most interest to *evo devo* practitioners are typically complex, lineage explanations that reside solely on the level of DNA sequences are insufficient to understand how developmental evolution transitioned from one state to the next: even though DNA sequence changes are of course an integral component of such an explanation it takes an understanding of how form comes into being—the *devo*—and how form-making is altered over generation,—the *evo*—to allow this level of causation in evolution to have explanatory power.

Second, *evo devo* contributes a novel type of causation that focuses on what we might call the degrees of freedom underlying developmental assembly, rather than simply the number and diversity of developmental component parts (Wagner 2000; Eble 2005). By recognizing that the nature of development is modular in developmental time, space, and regulation, *evo devo* is the first discipline to emphasize that evolutionary changes in aspects of this modular organization contribute critical degrees of freedom, or axes of variability, that enable and guide developmental evolution. Here the explanatory cause contributed by *evo devo* lies less with the discovery of the individual module: a module in it of itself contributes the same explanatory value as a gene might. Instead it comes with the discovery of the dimension within which a given modularity exists—space, time, cis-regulation—that contributes novel explanatory power, thereby enriching our understanding of what causes developmental evolution to unfold the way it does (Uller et al. 2018).

What Is Next? Current Conceptual Challenges to *Evo Devo*

Over the past 30 years, evolutionary developmental biology has provided context after context that establish development and evolution as both cause and effect of each other. Development is one of the many products of phenotypic evolution, which in turn is shaped by the nature of developmental processes. Viewed this way, *to build a phenotype requires development, while to evolve a change in phenotypes requires changes in the genetic basis of development*. At the same time *evo devo* is encountering its own challenges, both from within as well as from neighboring disciplines, which it must meet in order to remain relevant. I would like to close this chapter by highlighting the three challenges I consider most significant.

Too many genes, too little development Over the past 50 years, developmental biology has morphed into developmental genetics. Attending developmental biology meetings now one finds precious few talks or posters where the emphasis isn't on characterizing genes whose products and interactions contribute to the formation of some, preferentially experimentally very tractable, trait. *Evo devo* reflects the same trend and should perhaps be more aptly named *evolutionary developmental genetics*. *Evo devo* text books and courses are filled with examples of developmentally significant genes whose evolution has contributed in some way to important developmental changes in how traits are made. *But making a difference in a trait is not the same as making a trait*, especially not the complex traits *evo devo* is most concerned with (Keller 2010). Instead, traits are the products of developmental systems to which genes and their products contribute important interactants. Other products and interactions that are just as critical for trait formation emerge on other levels of biological organization as well, for example, through the communication among cells, or reciprocally inductive events among tissues, or the complex feedbacks commonplace among the component parts of organ system (Moczek 2015). While genes and genetic variation contribute to each of these as well, and therefore contribute to making such interactions reliably heritable, that relationship is not nearly as straightforward as that between a transcription factor and its binding site. Thus, while *evo devo* has managed to point us in productive directions as to how to better conceptualize and investigate the origins of diversity, novelty, and complexity, full realization of this goal will require a reorientation away from an understanding of traits and organisms as residing solely in genes and genomes, and toward an appreciation of traits as products of developmental systems. Viewed this way, *phenotypes emerge from developmental systems, whose evolution requires heritable changes in system functions*.

The contingent nature of development and developmental evolution: What, exactly, is environment? The proper functioning of all developmental processes ultimately depends on context. Context, in turn, is created by past developmental processes generating conditions for the next round of phenotype construction to take place. This constructive nature

of development is so ubiquitous we tend to overlook that it is of profound significance in ensuring the proper progression of development. Only more recently has it come into the focus of developmental biologists and *evo devo* practitioners that this context—or *environment*-constructing ability of developmental processes—does not end with where we conventionally assume the organisms itself ends: instead we now recognize that organisms, through their behavior, metabolism, and choices, actively and non-randomly also modify their external environment in ways that in turn feed back to affect their own fitness. Such *niche construction* blurs our conventional understanding of where organisms end and their environment begins, and opens up additional routes to adaptation and inheritance: organisms may no longer adapt solely by modifying their traits to suit environmental conditions but modify environmental conditions to suit their traits. Similarly, organisms no longer endow their offspring just with a set of genes but pass on to them everything from methylation states to transcripts, antibodies to symbionts, and territories to positions within a social hierarchy (Laland et al. 2015). Put together, the constructive nature of organismal functioning thus transcends many dimensions both internal and external to the organism. Viewed this way, *to develop is to interact with (and often construct) internal and external environmental states. Developmental evolution then requires alteration of these interactions in a heritable manner.* Integrating the study of the mechanisms and consequences of these interactions into its portfolio of research programs will greatly enhance the explanatory power of *evo devo*.

Microecoevodevo *Evo devo* is correct in its assessment that our ability to understand, reconstruct, and predict the evolution of complex traits will be impossible without an explicit developmental, phenotype-*constructing* perspective. But population genetics is also correct in its assessment that all genetic evolution is subject to the rules and constraints imposed by population biology. And, as emphasized above, future models must better integrate the simultaneously environment-dependent and -constructing nature of development and developmental evolution. How best to achieve this is unclear, but the quantitative frameworks that already exist in population genetics and niche construction theory on one side, and the increased appreciation of developmental symbioses and phenotypic plasticity within *evo devo*, offer good starting points to continue and deepen the necessary conversations.

Conclusion

The novel ways of thinking advanced by evolutionary developmental biology are providing powerful, new approaches to expand and, in part, correct our thinking on cause and process in evolution, thereby putting us in a position to resolve long-standing questions across diverse biological disciplines, in particular in evolutionary biology. *Evo devo* itself has grown tremendously in the recent past (reviewed in Moczek et al. 2015), and I expect

this transformation to further escalate as *evo devo* connects more thoroughly and thoughtfully with ecology, population genetics, and microbiology, at a time when an integrative and holistic understanding of evolutionary processes, and their causes and consequences, is more needed than ever.

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