CHAPTER 20

Variation and Developmental Biology: Prospects for the Future

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INTRODUCTION

Recent years have seen great strides in our understanding of organismal development. At the dawn of the twenty-first century, we have identified many key signaling pathways and mechanisms of gene regulation. We have also defined at a molecular level many of the phenomena identified by embryologists during the early and middle years of the twentieth century, including the organizer and limb polarizing activity of vertebrates and the maternal determinants of insect early patterning. It might seem that many of the biggest questions have been answered and that only the relatively minor details need to be elucidated.

In this review and prospectus, I argue that, in fact, many of the biggest questions for developmental biology still remain unanswered. Some of these questions explicitly concern variation. Other questions will be answered only with a deeper appreciation of variation in a developmental context. In both cases, a new incorporation of population thinking into developmental biology will need to be achieved.

Ernst Mayr introduced the concept of "population thinking" as one of the three major contributions made by Darwin (Mayr, 1976). Although the first two contributions—compelling demonstration of evolution and identification of natural selection as an evolutionary mechanism—have been widely celebrated, the third, replacing typological thinking with population thinking, had not been recognized. Mayr relates typological thinking to Platonic idealism, in which observed variability merely reflects an underlying natural "idea" or type. In this view, variation is simply noise with no inherent meaning; truth lies only in the types themselves and discontinuities between types represent the true order of the natural world.

By contrast, a population-based view of nature holds that variation is the true state of being: the uniqueness of individuals is real and it is the type that is an abstraction. Mayr argues that organisms can be described collectively only in statistical terms: means (and variances) are thus necessary abstractions if we are to summarize the true differences among individuals. Mayr was principally concerned with the ways in which typological and population thinking can influence our views of evolution, with a populational approach providing a clearly superior rationale for understanding organismal differences and how they have come into existence. The success of evolutionary biology following the neo-Darwinian synthesis, and its (implicit or explicit) embrace of population thinking, provides ample evidence for Mayr's assertion.

Developmental biology and one of its foundations, comparative morphology, have a long history of typological thinking (for recent reviews, see Amundson, 1998, 2000; Hall, 1999; Richardson *et al.*, 1999). The typological approach can be seen in Buffon's and Geoffroy Saint-Hilaire's idealistic morphologies, in which all forms were thought to derive from just one or a few types. The typological approach also is evident in the theories of Georges Cuvier's, which defined four "*embranchements*" into which all animals could be classified; variation within an *embranchment* merely reflected deviation from an ideal functional scheme, and life outside of *embranchments* was an impossibility. Although the perspectives of Saint-Hilaire and Cuvier differed dramatically in relating form to function, both held to notions of ideal types. Von Baer, who saw types in terms of embryonic form, rather than adult form, continued this tradition. Likewise, Owen defined the term "archetype" to represent an idealized form, and he proposed the now famous vertebrate archetype that has been reproduced frequently in textbooks. Finally, Haeckel's famous (or infamous) depiction of similarities among vertebrate embryos reflects an inherently typological view that sought to minimize the uniqueness of individuals or species while highlighting an idealized form (Richardson *et al.*, 1997, 1998).

The modern era of developmental biology has its roots in comparative morphology, the experimental embryology of Roux, and the genetics of Morgan, and as such represents a synthesis of several disparate disciplines. In this chapter, I suggest that the synthesis of modern developmental biology has remained essentially

typological in outlook, despite operating in parallel with the fundamentally populational neo-Darwinian synthesis. For developmental biology to address the remaining large questions before it, however, will require the adoption of an increasingly populational perspective. In the following sections, I suggest several of these questions, each relating to variation, and how a populational viewpoint will need to be accommodated.

I. MODEL ORGANISMS: EXPANDING THE FOLD

A key factor underlying the last 50 years of progress in developmental biology has been the emergence of a few, key "model organisms." Among these are the venerable fruit fly *Drosophila melanogaster* (aka "the Fly"), the nematode *Caenorhabditis elegans* ("the Worm"), the anuran amphibian *Xenopus laevis* ("the Frog"), the chicken *Gallus gallus*, the mouse *Mus musculus*, as well as more recent additions such as the mustard weed *Arabidopsis thaliana* and the zebrafish *Danio rerio*.

The use of model organisms has greatly facilitated advances through the resulting concentration of resources. Perhaps the most dramatic of resources is the availability of sequence data for the complete or nearly complete genomes of each of these models. Although whole genome sequences and other genomic resources (e.g., expressed sequence tags, gene chips) have appeared only in the last few years, researchers working on the model organisms have long enjoyed the availability of molecular probes, normal tables of development, standardized protocols for molecular biology and histology, methods for embryological and genetic manipulation, and specialized stocks of various sorts, including mutant strains and inbred lines.

Model organisms also have afforded us vast intellectual resources. Although the primary literature on model organism development is vast and continually expanding, less appreciated is the unpublished, communal knowledge, or "lore," about these organisms ("How does one fertilize fish eggs in vitro?" "How much aeration do the frogs really want?" "What is the best way to explant a chicken neural tube?"). Together, these bodies of knowledge have allowed extraordinary progress in dissecting many basic mechanisms of development.

Today's model organisms have been chosen primarily for their convenience. In some cases the organisms were readily (or even too readily) available. In other instances they were chosen because they are easy to breed or to rear. Sometimes optical clarity, invariant cell lineage, or both have been factors, and almost always, speed of development has been important. Model organisms have not been singled out for study because they are likely to be representative of particular groups or modes of development. Nevertheless, it has often been assumed that what occurs during the development of the premier model organisms can be generalized to others of their genus, order, or phylum. In many cases this is true. In some it is not.

As noted by others (Kellogg and Shaffer, 1993; Bolker, 1995; Metscher and Ahlberg, 1999), the very features that make our model organisms attractive to study can also make our inferences suspect when we try to generalize our findings more broadly. For example, rapid development alone likely entails a host of specializations (Hadfield, 2000). More typically, however, model organisms are like any other organisms in exhibiting a host of traits, some of which are relatively derived and some of which are relatively primitive for their group. Thus, even ignoring the specific features that have made some model organisms the favorites of developmental biologists, some unique derived features will be encountered simply by chance. Studies of amphibian gastrulation and *Drosophila* early pattern formation illustrate the sometimes surprising ways in which development of our model organisms can differ from their nonmodel relatives.

The workhorse model amphibian is *X. laevis*, and pioneering studies by Keller and colleagues have elucidated many of the morphogenetic behaviors occurring during gastrulation and mesoderm formation in this species (Keller *et al.*, 2003). Fate mapping (Keller, 1975, 1976) demonstrated that prospective mesoderm resides only within a deep layer of involuting cells at gastrulation. As gastrulation proceeds, the prospective mesodermal cells already are situated within the "correct" tissue layer, between the roof of the archenteron and the overlying ectoderm. In contrast, early studies showed that most frogs, as well as salamanders, have prospective mesoderm on the surface of the early gastrula, in addition to cells in the deeper layer. As gastrulation proceeds in these embryos, the initially superficial cells find themselves lining the roof of the archenteron and must then ingress secondarily to join the rest of the mesoderm (Vogt, 1929; Purcell and Keller, 1993). A potential transitional form has been observed in *Hymenochirus*, the closest relative of *Xenopus* studied to date, with prospective mesodermal cells ingressing from the archenteron as a sheet rather than as individual cells (Minsuk and Keller, 1996). Thus a fundamental aspect of vertebrate development—how the germ layers arise during gastrulation—differs substantially between *Xenopus* and most other amphibians.

Perhaps the best understood developmental patterning system is that of anterior–posterior axis determination in *D. melanogaster*. Whereas early embryological manipulations with other insects pointed to the existence of maternal determinants in the early embryo (Kalthoff and Sander, 1969; Sander, 1975), it was the work of Driever and Nusslein-Volhard that identified the molecular bases for these events (Driever and Nusslein-Volhard, 1988a,b). Of primary importance is *bicoid*, which is deposited as a maternally derived mRNA at the anterior end of the developing *Drosophila* oocyte. Major functions of *bicoid* protein are to repress the translation of *caudal* mRNA, at the posterior end of the embryo, and to upregulate the transcription of the gap gene, *hunchback* (Ephrussi and Johnston, 2004). Embryos that are mutant for *bicoid* lack anterior head and thorax. In the relatively closely related fly, *Megaselia*, a role for *bicoid* is evident not only in head and thorax development, but also in anterior abdomen formation (Stauber *et al.*, 2000).

Despite the pivotal role of *bicoid* in establishing anterior–posterior pattern in *Drosophila*, this is not the case for other insects or even more distantly related flies. The *bicoid* gene arose by duplication of an ancestral *Hox3/zerknüllt* locus in the lineage leading to cyclorrhaphan flies, of which *Drosophila* and *Megaselia* are members. In these flies, *bicoid* is expressed maternally, whereas its paralogue *zerknüllt* is expressed zygotically. In more basal dipterans that lack *bicoid*, the single *Hox3/zerknüllt* locus is expressed both maternally and zygotically and is likely to serve functions now divided between *bicoid* and *zerknüllt* in cyclorrhaphans (Stauber *et al.*, 2002). Beyond the dipterans, the flour beetle *Tribolium castaneum* lacks a *bicoid* gene, but *orthodenticle* and *hunchback* (gap genes downstream of *bicoid* in *Drosophila*) together fulfill an equivalent function (Schroder, 2003; see also Wimmer *et al.*, 2000). Relative contributions of maternal and zygotic genes to anterior–posterior patterning in the wasp *Nasonia* are also likely to differ substantially from *Drosophila* (Pultz *et al.*, 1999). Thus a basic feature of early patterning differs dramatically in its execution across insects.

These examples reveal how a focus on a few model organisms can speed progress toward an understanding of mechanism, but also how a typological view of these mechanisms could lead to erroneous conclusions about their generality. Additional examples of developmental convergence and parallelism (in the following text) provide ample evidence for the importance of studying mechanisms across multiple taxa even within the same order or family. When studies of basic developmental mechanisms are conducted within an explicitly phylogenetic context, fresh insights into the generality of developmental mechanisms seem always to result.

Early embryologists studied a far more diverse range of organisms than most developmental biologists currently employ. Perhaps most dramatically missing from the mainstream are the lophotrochozoans. Modern molecular phylogenies reveal three major clades of animals: Deuterostomia, Ecdysozoa, and Lophotrochozoa (Adoutte *et al.*, 2000; Balavoine *et al.*, 2002; Ruiz-Trillo *et al.*, 2002; Anderson *et al.*, 2004). All of the commonly studied model organisms fall within the deuterostomes (sea urchin, zebrafish, frog, chicken, mouse) or ecdysozoans (*Drosophila*, nematodes). By contrast, the lophotrochozoans (leeches, mollusks, and possibly flatworms) were studied extensively by early workers and then largely fell out of favor. For example, snail embryogenesis was studied by Conklin at the turn of the last century (Conklin, 1897, 1902) and has been studied sporadically since then (Freeman and Lundelius, 1982), but modern advances in molecular and cellular biology typically have not been applied to understanding the development of these organisms (for a notable exception, see: Lambert and Nagy, 2001). Nevertheless, new resources are becoming available for working even with "nonmodel" species (Voss *et al.*, 2001; Newmark and Sanchez Alvarado, 2002; Tessmar-Raible and Arendt, 2003) that should substantially facilitate studies of basic developmental mechanisms across a wider swath of the animal kingdom. A recognition that

current biomedical models are not types, or even averages, of their respective groups, but merely sample species drawn for particular purposes, will be essential for a more complete understanding of development.

II. ECOLOGICALLY SIGNIFICANT DIFFERENCES IN FORM BETWEEN SPECIES

A basic goal of developmental biology is to understand how a fertilized egg gives rise to an adult body. This transformation involves increasing complexity through the processes of pattern formation, morphogenesis, differentiation, and growth. To date, we know a great deal about these processes during early embryogenesis, when the body axes and germ layers are established, when the organ rudiments are forming, and when the organism is interacting only minimally with its environment. Yet we still understand relatively little of the mechanisms responsible for the precise forms of larvae, juveniles, and adults that interact extensively with their environments. Whether the shapes of spicules in a sea urchin pluteus, the color pattern of a caterpillar, the skull shape of an owl, or the growth habit of an oak tree, we typically do not know the genes and cell behaviors underlying the expression of these traits. A major remaining goal for developmental biology is to understand the mechanisms responsible for juvenile and adult form, and by extension, how differences in these mechanisms generate variation in form across taxa.

Perhaps of greatest interest are the developmental bases for interspecific differences that are likely to have adaptive significance. Such analyses can be conducted at several phylogenetic levels. For example, the turtle shell represents an evolutionary innovation having clear adaptive significance, and recent analyses are beginning to identify the signaling pathways underlying shell development (Gilbert *et al.*, 2001; Rieppel, 2001). Comparisons with squamate reptiles and birds will allow new insights into the developmental and evolutionary origins of this structure.

Besides true novelties, the developmental bases for adaptive modifications to existing form between major groups have started to be dissected with some success. Examples come from studies of arthropod and vertebrate appendages. In most arthropods, thoracic appendages are used for locomotion, but in several groups of crustaceans, anterior thoracic appendages have become specialized for food handling. The evolutionary appearance of these transformed legs, or maxillipeds, is associated with losses in the expression of the Hox genes from anterior thoracic segments (Averof and Patel, 1997). Variation in arthropod Hox gene expression has been associated with other morphological differences as well (Averof and Akam, 1995; Popadic *et al.*, 1998; Abzhanov and Kaufman, 2000).

More recently, factors determining appendage number in arthropods have been studied, with a focus on evolutionary changes in the function of Ultrabithorax (Ubx) protein. In *Drosophila*, thoracic limb outgrowth requires *Distal-less* (*Dll*)

expression, but in the abdomen where limbs do not form, *Dll* is repressed by Ubx. If Ubx is ectopically expressed in the thorax, it represses *Dll* there as well, and legs do not form (Gonzalez-Reyes and Morata, 1990; Mann and Hogness, 1990; Vachon *et al.*, 1992). Thus *Dll* expression is correlated with limb development in *Drosophila* and a diverse array of other species as well (Panganiban *et al.*, 1994, 1997; Panganiban, 2000). By contrast, crustaceans have abdominal legs and abdominal *Dll* expression—but also abdominal Ubx expression—suggesting a difference in the way Ubx interacts with *Dll* in this group, as compared with *Drosophila*. Ectopic expression in *Drosophila* embryos of native crustacean Ubx and chimeric proteins reveals the presence of a carboxy terminal Ubx domain that allows for the conditional repression of *Dll* in crustaceans, rather than the constitutive repression of *Dll* in *Drosophila* (Ronshaugen *et al.*, 2002). Thus a difference in Ubx function is associated with the absence of abdominal limbs in *Drosophila* and the presence of abdominal limbs in crustaceans. Analyses of phylogenetically more basal Onychophora (which have legs on each body segment, including the abdomen) reveal that an onychophoran Ubx completely fails to repress *Dll* expression because it lacks key amino acid residues (Grenier and Carroll, 2000; Galant and Carroll, 2002) (though this difference is not likely to be functionally significant in generating the many limbs of onychophorans per se, in that *Ubx* and *Dll* expression do not appear to overlap in leg-bearing segments [Grenier *et al.*, 1997]).

Several studies of vertebrates also have been informative in understanding the evolution of adult form across relatively divergent taxa. For example, a recent study of the shark *Scyliorhinus canicula* may reveal a transitional stage in the evolution of limbs from fins (Tanaka *et al.*, 2002). In tetrapods and teleosts, *Tbx5* and *Tbx4* play essential roles in specifying the form of anterior and posterior appendages, respectively. In *Scyliorhinus*, both of these genes are expressed in a manner reminiscent of other vertebrates. A different situation is observed for *sonic hedgehog* (*shh*). In tetrapods, *shh* is expressed in the posterior margin of the limb mesoderm; Shh protein exhibits polarizing activity during limb development and contributes to limb outgrowth by a feedback loop involving bone morphogenetic protein (Bmp) and fibroblast growth factor (Fgf) signals (Riddle *et al.*, 1993; Laufer *et al.*, 1994; Niswander *et al.*, 1994; Khokha *et al.*, 2003). In the *Scyliorhinus* embryo, however, *shh* transcript is conspicuously absent from the developing fin, though it is expressed in other locations (e.g., notochord and floor plate). Thus it is conceivable that a change in *shh* expression is associated with transition from a sharklike, cartilaginous fin to the tetrapod limb (though expression of *shh* in zebrafish fin buds [Krauss *et al.*, 1993; Akimenko and Ekker, 1995] suggests an alternative scenario in which *shh* expression in developing appendages is ancestral and has been lost from *Scyliorhinus*, rather than gained in tetrapods).

Finally, analyses of patterning genes also shed light on the evolution of limblessness in snakes (Cohn and Tickle, 1999). In limbed tetrapods, Hox gene anterior expression boundaries correlate with the morphological thoracic–cervical

transition, where the forelimbs will develop (Burke *et al.*, 1995). In python embryos, however, thoracic Hox genes are expressed throughout the trunk, and limbs do not form anteriorly, suggesting that limb axial repatterning to a thoracic identity has reduced or eliminated the anterior limb field. Although vestigial limb buds are present posteriorly, these buds lack *shh* expression in the mesoderm as well as a morphologically identifiable apical ectodermal ridge with *Fgf8* expression, which is required in other tetrapods for limb outgrowth. Thus changes in axial patterning and loss of the Shh–Bmp–Fgf regulatory loop appear to be associated with limb loss in pythons and presumably the ancestors to modern snakes.

The preceding examples analyzed variation in developmental patterning at relatively deep phylogenetic levels. Although such comparisons are necessary for some of the most dramatic evolutionary transformations, such as the loss and gain of limbs, these comparisons also entail several inherent difficulties. For example, distantly related taxa will have accumulated a variety of developmental differences that are independent of the evolutionary transformation in question, making it more difficult to assess what is causally relevant and what is not. For this reason, studies of variation in developmental mechanisms between closely related species sometimes can provide insights that broader comparisons cannot. For instance, studies of pigment pattern formation in salamander larvae identified presumptively ancestral cell–cell interactions required for stripe formation in several species, but novel and redundant mechanisms in one of these species, in which stripes are somewhat more distinctive (Parichy, 1996a,b, 2001). Such developmental elaborations may represent early stages in the evolution of a trait associated with new selective consequences for the expression of that trait. Thus analyses of variation in cellular interactions across closely related species can provide insights into how initially simple characters are molded through selection into more complex and specialized adaptations.

At a molecular level, another problem inherent to comparisons across deep phylogenetic divides is that such comparisons can be limited to known genes and pathways. For example, extensive previous work on limb patterning and morphogenesis made it possible to compare the expression and activity of candidate genes across taxa. In some instances, however, adaptive differences between species are likely to result from changes in genes or pathways that have not yet been studied in model organisms. One approach to identifying "new" genes as well as "old" genes is the genetic analysis of species or populations that are morphologically different but sufficiently closely related to interbreed. Several analyses of teleost fishes highlight these strategies.

One system that has been employed for analyzing variation among closely related species is the adult pigment pattern of *Danio* fishes, including the zebrafish *D. rerio* (Parichy, 2003). Pigment patterns have clear adaptive significance as they are used for mate recognition and mate choice, as well as predation avoidance and schooling (Endler, 1983; Houde, 1997; Couldridge and Alexander, 2002;

Engeszer *et al.*, 2004). *Danio* fishes exhibit a diverse array of patterns, including horizontal stripes, vertical bars, spots, and uniformly dispersed pigment cells. Moreover, pigment patterns exhibited by many *D. rerio* mutants resemble the naturally occurring patterns of other species, suggesting the affected loci as *a priori* candidate genes for contributing to pattern differences across taxa. Since hybrids can be produced between *D. rerio* and other species, and these hybrids typically have horizontal stripes like *D. rerio*, complementation tests can be used to assess whether the same genes isolated as *D. rerio* mutants might contribute to interspecific differences as well. This approach showed that one mutant *panther*, which lacks stripes, fails to complement in crosses with *D. albolineatus*, which also lacks stripes. Molecular cloning and analysis revealed *panther* to be a *Danio* orthologue of the *fms* gene, which encodes a receptor tyrosine kinase expressed by pigment cells in danios, but not previously known to have a role in pigment pattern formation (Parichy *et al.*, 2000; Parichy and Johnson, 2001; Quigley *et al.*, 2005; see also Long *et al.*, 1996; Sucena and Stern, 2000). This example illustrates how forward genetics in a model organism, coupled with interspecific genetic analysis, can be used to find novel genes associated with ecologically significant variation between taxa.

Two other groups of fishes that have been examined in this context are cichlids and sticklebacks. African cichlids have long been the premier model for adaptive radiations, with more than a thousand species arising over the past million years in Lakes Malawi and Victoria (Allender *et al.*, 2003). Despite their recent origins, these species differ dramatically in jaw morphology, color pattern, and behavior. Quantitative trait locus (QTL) mapping has started to define chromosomal regions that contribute to these differences (Albertson *et al.*, 2003a,b; Streelman *et al.*, 2003).

Stickleback fishes also have a long history of evolutionary, ecological, and behavioral research (Peichel and Boughman, 2003), and different populations exhibit a variety of morphological differences, particularly in dermal armor plating and internal osteological characters. One such trait is the pelvis, which is dramatically reduced or absent in some populations. Construction of a genetic map for sticklebacks has allowed the identification of chromosomal regions as well as candidate genes that contribute to this variation (Peichel *et al.*, 2001). In a recent analysis, pelvic reduction in a freshwater stickleback population was shown to segregate primarily as a single Mendelian factor, and QTL mapping associated a large fraction of the segregating variance with a single chromosomal region. In this instance, mapping of candidate genes based on studies of limb development in model organisms placed *Pitx1* within the interval identified by the quantitative trait locus. Subsequent analyses suggest that a site-specific loss of *Pitx1* expression from the prospective pelvic region is causally relayed to pelvic reduction in this population (Shapiro *et al.*, 2004).

Recent studies of danios, cichlids, and sticklebacks highlight the potential for analyses of closely related species to reveal the genetic and developmental bases

for adaptive differences in adult form. Moreover, the many QTL and candidate gene studies performed to date are starting to provide insights into the sheer number of genes contributing to morphological differences between species and populations (Voss and Shaffer, 1997; Doebley *et al.*, 1997; Long *et al.*, 1998; Frary *et al.*, 2000; Kopp *et al.*, 2003). Typically, anywhere from one to several major effect loci have been identified. These studies are limited in their power to detect loci of very small effects, and it is difficult to judge how many studies might have failed to detect major genes owing to publication biases. Nevertheless, the frequent identification of loci explaining substantial proportions of phenotypic variance suggests a more important role for fewer genes of large effect than was assumed by standard evolutionary genetic models, which assumed many genes of small effect (Orr and Coyne, 1992). Thus we are starting finally to understand how many genes contribute, at least substantially, to standing morphological differences between species and populations.

At least three major challenges remain, however. A first, largely technical challenge will be the development of additional methods for identifying genes and pathways contributing to variation between species. Although hybrid analyses and QTL mapping can be useful in some cases, many of the most interesting morphological and developmental differences are between species that are closely related, but not so closely related that viable or fertile hybrids can be produced for genetic analysis (e.g., variation in beak morphology [Schneider and Helms, 2003]). For still other species, genetic approaches might be possible in principle but too difficult to accomplish in practice. Development of additional, unbiased approaches to identifying new genes and pathways underlying interspecific differences will make a major contribution to this area. Conceivably, microarray technologies will be useful in this regard, when coupled with appropriate screening strategies and statistical analyses to control for phylogenetic divergence (Rifkin *et al.*, 2003).

A second, largely conceptual challenge will be linking genes to morphological outcomes. To understand how variation in developmental mechanisms generates variation in adult form, identification of genetic differences is only useful to the extent that these differences are placed in the context of defined cellular behaviors, such as proliferation, death, migration, and differentiation. Bridging the morphogenetic gap between genes and phenotypes is a major challenge even for studies of embryogenesis using model organisms. Doing so to understand differences in adult form between species requires a populational approach at multiple levels of biological hierarchy, from genes and their activities, to cells, to tissues, and to organs.

Finally, a third technical and conceptual challenge will be to relate variation between populations or species to variation within populations themselves. Simply because we can detect loci currently associated with moderate to large phenotypic differences, this association need not imply that the differences initially evolved via

mutations having moderate or large effects or even by mutations at the identified loci. In *Danio* fishes, for example, a loss of stripes in *D. albolineatus* was associated with variation at the *fms* locus (Parichy and Johnson, 2001). Although this may reflect a saltational loss of stripes owing to a major effect mutation in *fms* itself, it could also reflect more gradual changes at *fms*, evolutionary changes in cellular requirements for *fms*, or changes in other *fms* pathway genes (Quigley *et al.*, 2005). Elucidating the relationships between interspecific and intrapopulational variation will require a deeper understanding of how allelic differences within populations influence trait expression during development.

III. HOW MANY WAYS TO MAKE A PHENOTYPE: DEVELOPMENTAL VARIATION AND MORPHOLOGICAL SIMILARITY

Besides explaining the mechanistic bases for differences in form across species, a major unresolved question is the extent to which the same phenotype in different species arises by different—or the same—underlying mechanisms. Developmental variation sometimes can be identified even for traits that have a common evolutionary origin. Differences in mesoderm formation among anuran amphibians (as noted previously) are one example in which ancestral morphogenetic mechanisms have been altered in the lineages leading to *Hymenochirus* and *Xenopus*. Another classic example is Meckel's cartilage, which is induced through an epithelial–mesenchymal interaction that employs different epithelial tissues in different vertebrates (Hall, 1984). Extensive analyses of nematode vulval development have also identified very different genetic and cellular mechanisms across species (Jungblut and Sommer, 2000; Sommer, 2001), and cellular ablation studies in leeches have shown interpopulation differences in the earliest cellular interactions that specify cell fates (Kuo and Shankland, 2004). Finally, superficially similar adult stripes of zebrafish and its close relative *D. nigrofasciatus* nevertheless arise with very different contributions from temporally distinct populations of larval and adult melanophores (Quigley *et al.*, 2004).

The extent of cryptic developmental variation is especially relevant to cases of homoplasy, in which organisms look the same for reasons other than descent from a common ancestor. Typically, independent origins of a trait have been termed "convergence" if arising by different mechanisms, or "parallelism" if arising by the same mechanisms (Wake, 1991; Hodin, 2000). These alternative modes have profound implications for our understanding of developmental evolution.

If homoplasy consistently arises through different mechanisms (convergence), this would imply there are few limits on how developmental mechanisms can evolve. Alternatively, if homoplastic evolution typically occurs through the same

mechanisms (parallelism), this would suggest that some developmental pathways are more likely to be modified than others. Put another way, repeated instances of parallelism argue that some kinds of mechanistic variants are more likely to arise in populations (and so be available for selection) than others. Thus some pathways of evolutionary change would be more likely, even though multiple phenotypic solutions might be equally adaptive in the face of selection. Assessing the mechanistic bases of homoplasy thus provides crucial data for resolving debates over the importance of developmental biases (or "constraints") during morphological evolution (Maynard Smith *et al.*, 1985).

Although many examples of homoplasy have been identified and may hint at underlying mechanism (Wake, 1991; Huber *et al.*, 2000; Parra-Olea and Wake, 2001; Dowling *et al.*, 2002; Wray, 2002; Santos *et al.*, 2003; Smith and Johanson, 2003), we typically do not know the genetic or cellular bases for the traits in question. Recently, however, several studies have provided evidence at the mechanistic level. For example, repeated reductions in the numbers of larval trichomes in *Drosophila* are associated with changes at the *shaven baby* locus (Sucena *et al.*, 2003). Genetic analyses of sticklebacks also have identified common bases for the repeated evolution of pelvic reduction (Shapiro *et al.*, 2004) as well as armor plate reduction (Colosimo *et al.*, 2004; Cresko *et al.*, 2004). Finally, some particularly illuminating examples of mechanisms come from studies of pigmentation. Dark or "melanistic" phenotypes have evolved repeatedly and independently in both birds and mammals, and recent analyses reveal that many of these instances are associated with activating amino acid substitutions in the melanocortin receptor, Mc1r, which functions to promote pigment synthesis (Theron *et al.*, 2001; Eizirik *et al.*, 2003; Mundy and Kelly, 2003; Nachman *et al.*, 2003; Mundy *et al.*, 2004). Together, these examples reveal a striking degree of evolutionary parallelism that would not have been predicted by classical quantitative genetic models of evolutionary change (Barton and Turelli, 1989; Falconer and Mackay, 1996).

By contrast, other studies identify evolutionary convergence in developmental mechanisms. For example, some studies of melanism in amniotes (Nachman *et al.*, 2003), as well as pigment pattern evolution in *Drosophila* (Wittkopp *et al.*, 2003), reveal different genetic bases across species. Likewise, metamorphic failure, or paedomorphosis, has evolved repeatedly and independently in salamanders, and interspecific hybridization studies support a model in which different genetic changes are responsible in different phylogenetic lineages (Voss and Shaffer, 1996).

Thus both evolutionary convergence and parallelism in developmental mechanisms occur, and it remains to be seen what their relative frequencies might be. The answer to this question will dramatically impact our understanding of both developmental mechanisms and their evolution. Such answers will come only from developmental studies of homoplasy in an explicitly phylogenetic context, requiring a populational perspective on the nature of developmental mechanisms and how they can vary.

IV. INTRASPECIFIC DEVELOPMENTAL VARIATION: CANALIZATION AND DEVELOPMENTAL PLASTICITY

The preceding sections have focused on variation in developmental mechanisms across species or populations that may—or may not—be causally related to variation in form. An equally important, remaining problem in developmental biology concerns the nature of developmental variation within populations. Outside of developmental evolutionary biology (or evolutionary developmental biology, depending on one's predilections [Hall, 2000; Gilbert, 2003]), very few developmental biologists concern themselves with variation in cell behaviors and gene activities across species, let alone among individuals within populations. Nevertheless, such variation exists and determining its causes and consequences will be critical for progress both in biomedical and evolutionary research.

Variation in phenotype results from genetic influences, environmental influences, and interactions between the two (Falconer and Mackay, 1996; Lynch and Walsh, 1998). Evolutionary quantitative genetic models have provided a framework for understanding the sources of phenotypic variation, and such models typically have been applied to adult form (or behavior). Nevertheless, the same principles hold for developmental phenotypes, and several studies have examined the statistical bases of morphological variation at different stages of development (Blouin, 1992; Phillips, 1998; Watkins, 2001). Others have sought to provide a framework for partitioning this variance into components associated with genetic and environmental effects on cell behaviors and gene activities (Riska, 1986; Slatkin, 1987; Atchley and Hall, 1991; Cowley and Atchley, 1992). Such statistical approaches have the advantage of being readily incorporated into evolutionary genetic theory. It has not yet been possible to bridge the gap empirically between statistical estimates of quantitative genetics parameters and underlying developmental mechanisms. Nevertheless, such models serve a valuable heuristic purpose in formally identifying the potential sources of variation in developmental mechanisms. In the following discussion, I consider what we know and do not know about genetic and environmental sources of intrapopulational variation in developmental mechanisms.

As is true for interspecific and interpopulation variation in development, intrapopulation variation may or may not have morphological consequences. Understanding why some differences in gene activities and cell behaviors influence morphology while others do not is perhaps the single greatest challenge facing developmental biology in the future. In this regard, an essential piece of information is the extent of functionally significant standing allelic variation for developmentally relevant loci. For model organisms including human, these data are rapidly being compiled in the form of single nucleotide polymorphisms catalogued in the National Center for Biotechnology Information databases: (http://www.ncbi.nlm.nih.gov). Thus there is now the potential for systematically surveying intraspecific variation in coding as well as noncoding regions of the genome. Moreover, these data can

sometimes be associated with major phenotypic effects, particularly in the databases of human genetic disease syndromes (e.g., "Online Mendelian Inheritance in Man"). Thorough surveys of these sorts of data will provide new insights into the likelihood that genetic variants in coding or noncoding sequences have phenotypic effects (Rockman and Wray, 2002; Genissel *et al.*, 2004).

A long-standing observation in developmental genetics is the resistance of development to minor genetic and environmental perturbations, a phenomenon known as "canalization" (Waddington, 1942, 1957, 1975). One example of canalization is the insensitivity of most loci to dosage effects, resulting in the recessivity of many mutant alleles. Outside of mutant laboratory stocks, support for the existence of substantial variation in gene product abundance or activity comes from several sources. For example, microarray analyses of gene expression have revealed extensive variation in transcript abundance among individuals within populations (Oleksiak *et al.*, 2002) and between inbred strains (Pavlidis and Noble, 2001). In principle, such differences can arise from both *cis*-acting and *trans*-acting regulatory variation. Demonstration of substantial *cis*-acting regulatory variation comes from recent analyses of allele-specific transcript abundance in heterozygous individuals: 6–18% of surveyed loci exhibited significant differences in transcript abundance between alleles, typically with 1.5–3-fold imbalances (Cowles *et al.*, 2002; Bray *et al.*, 2003; Pastinen *et al.*, 2004). In the face of such variability, understanding how canalization arises—and why sometimes it fails (see the following text)—are fundamental questions if we are to understand how development translates genotypes into phenotypes.

One explanation for canalization in the face of genetic perturbation comes from inherent properties of developmental systems. For example, mathematical analyses of metabolic pathways have been applied to understand the nature of dominance and recessivity (Kacser and Burns, 1981; Dykhuizen *et al.*, 1987). Considering linear pathways of gene products, the sensitivity of the outcome to variations in the activity of any one pathway member diminishes as the total number of pathway members increases. Thus the more complex (or longer) the pathway, the less sensitive it is likely to be to minor perturbations at each step. Analyses of evolutionary change in human and mouse metabolic genes further supports a model in which increased network complexity is associated with increased canalization (Kitami and Nadeau, 2002).

A mathematical approach recently has been applied to understanding networks of interacting developmental genes as well. In two remarkable papers, the networks of genes comprising the *Drosophila* segment polarity and neurogenic networks have been modeled (von Dassow *et al.*, 2000; Meir *et al.*, 2002). These systems were chosen because of the extensive empirical data ordering genes within these pathways. Nonlinear equations were used to describe the interactions among gene products, and variations in their associated parameters were tested for their ability to produce the expected developmental outcome (i.e., simulated cells expressing

the correct genes in the correct places). The major finding from these models is the extraordinary robustness of the simulated pathways: Even very large deviations in gene product concentrations and activities were readily accommodated. Empirical analyses of Bmp signaling in *Drosophila* further support the robustness of such developmental genetic networks (Eldar *et al.*, 2002). Thus a classical view of gene regulation in terms of binary switches, with genes either on or off, may be a substantial (and typological) oversimplification. Rather, we may need to view even the most impressive and complex of resolved genetic pathways (Davidson *et al.*, 2002a,b) as merely rarified descriptions of interactive networks having substantial quantitative and stochastic components.

A second explanation for canalization of developmental mechanisms comes from studies of Hsp90 in *Drosophila* and *Arabidopsis* (Rutherford and Lindquist, 1998; Queitsch *et al.*, 2002; Sangster *et al.*, 2004). Hsp90 acts as a chaperone to ensure correct protein folding particularly in response to stress. Studies of Hsp90 mutants reveal dramatically increased frequencies of developmental anomalies in a variety of traits. Thus Hsp90 suppresses the phenotypic expression of underlying genetic variation, presumably in part through its function as a chaperone and apparently also through chromatin modification (Sollars *et al.*, 2003). A recent analysis identifies the epidermal growth factor receptor gene (*Egfr*) as one of the loci for which cryptic variation is buffered by Hsp90 in nature (Dworkin *et al.*, 2003). It will be especially interesting to see what other genes contribute to canalization of phenotypes in a manner analogous to Hsp90, as well as the extent to which variation at such loci contributes to corresponding variation in canalization.

When developmental canalization breaks down, it can do so in response to genetic or environmental influences. Genetically, this can reflect either novel combinations of existing alleles or the introduction of new allelic variants by mutation. That some allelic combinations simply "work" better than others in the face of genetic perturbations is reflected in the common observation of genetic modifiers to laboratory-induced mutations: For the same mutant locus, the severity of phenotypic effects can differ drastically across genetic backgrounds (Rhim *et al.*, 2000; Nadeau, 2001; Taddei *et al.*, 2001; Slavotinek and Biesecker, 2003). Variable penetrance and expressivity of human disease syndromes presumably also reflects the particular combinations of alleles across loci in individual genomes. In the context of mathematical analyses of gene networks (Meir *et al.*, 2002; von Dassow *et al.*, 2000), we can perhaps view such differences in phenotype as a failure of canalization, occurring in individuals having genomes already lying on the fringes of acceptable parameter space. Similarly for newly arising mutations, we can imagine that some alleles will impact the network of interacting genes significantly enough to affect trait expression. Predicting the phenotypic consequences of such perturbations will require a deeper understanding of morphogenetic mechanisms themselves and how variation in these mechanisms depends on the underlying developmental genetic networks. Novel approaches to this problem will allow a fuller characterization

of phenotype space that is accessible through short-term evolutionary change (e.g., phenotypes that are one and two mutational steps away from "wild-type," [Dichtel-Danjoy and Felix, 2004]). Such an advance would complement statistical approaches to predicting the effects of standing genetic and phenotypic variation to short-term evolutionary change (Schluter, 1988; Falconer and Mackay, 1996; Lynch and Walsh, 1998; Arnold *et al.*, 2001).

A final important aspect of developmental variation within populations is the breakdown of canalization in response to environmental factors. This responsiveness of development to the environment is typically referred to as "plasticity," and an enormous body of literature has examined such plasticity in an ecological and evolutionary context (Roff, 1992; Stearns, 1992; Callahan *et al.*, 1997; Price *et al.*, 2003; Relyea, 2004). Plasticity has been profoundly understudied by mainstream developmental biology. Nevertheless, there are many examples of plasticity affecting development rate as well as the development of morphological traits that beg for thorough mechanistic analyses. For instance, many amphibians undergo a metamorphosis, and the timing of this larval to adult transformation can be highly dependent on environmental stresses such as temperature and crowding (Wilbur and Collins, 1973; Parichy and Kaplan, 1992). Interactions between stress hormones and thyroid hormones appear to be important, but much more work at the level of molecular mechanisms is required (Denver, 1998). Similarly, individuals of some species of salamanders can choose between metamorphosis to a terrestrial adult form or the acquisition of sexual maturity in an aquatic, otherwise larval form (Ryan and Semlitsch, 1998). Such facultative metamorphic failure, or paedomorphosis, is only beginning to be analyzed at the level of developmental and genetic mechanisms (Voss *et al.*, 2003; Voss and Smith, 2005). Finally, a wide range of discrete alternative morphologies are inducible by environmental stimuli, including predator-induced development of spines in *Daphnia*, large-mouthed cannibalistic morphs in salamander larvae, and facultative wing development in aphids (Westeberhard, 1989; Hoffman and Pfennig, 1999; Nijhout, 1999; Barry, 2000). Instances of developmental plasticity pose outstanding challenges and rewards, for furthering our understanding of how developmental regulatory and morphogenetic mechanisms influence variation within populations and individual life cycles.

V. CONCLUSIONS

In this review I have surveyed developmental variation at several levels of biological organization, from deep phylogenetic divides to variation within individuals. In some instances, variation in developmental processes results in phenotypic variation with clear impacts on individual fitness, and in other instances, no phenotypic effects are discernable. At each of these levels, determining the causes and consequences of this variation will be of tremendous importance for our understanding

of basic developmental mechanisms, human health and disease, and organismal evolution. To achieve these goals, developmental biology will need to more fully embrace populational perspectives on biological organization and variation.

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