CHAPTER 7

Developmental Origins of Variation

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INTRODUCTION

Writing about the origin of developmental variation is an intriguing challenge for someone who has long been interested in the flip side of developmental variation, namely canalization. Both variation and canalization are of paramount interest because of their relationship to the patterns and processes of evolution. With respect to developmental variation, did Darwin not get it right? "Descent with modification" means that similarities between taxa are the result of common ancestry, and differences are the result of modifications in development wrought

by hereditary changes. Although the problems inherent in identifying the paths of descent and recognizing modifications are many (see Hall, 2003, for a recent discussion of these issues), the emphasis on the importance of hereditary change leads to the conclusion that evolution results from the "selectable developmental variation" (West-Eberhard, 2003). Mutational processes propose variations, and selection disposes of them.

Although, historically, developmental variation and selection may have been twin pillars of evolution, the focus in the twentieth century was on selection. The reason for this has been discussed in a compelling way by West-Eberhard (2003) who points out that developmental variation took a back seat to selection when it was deemed to threaten another sacred tenet of neo-Darwinism, gradualism in evolution. Whatever the historical reasons for its temporary fall from view, the resurgence of interest in evolutionary developmental biology places developmental variation and its origins at the center of any understanding of the nature and dynamics of evolution. Conventional wisdom suggests that the origin of developmental variation results from mutational or environmental perturbations. Few can doubt that genetic variation exists in abundance and plays an important role in evolution. Intuitively, we appreciate genetic variation every time we observe denizens of city parks; both the variety of plumage color patterns in pigeons and the variety of humans we encounter suggests that genetic variation is common. Indeed, the chief message of twentieth-century population genetics was that there is so much genetic variation in natural populations, much of it hidden from gross phenotypic expression, we were hard put to explain how it was all maintained (Franklin and Lewontin, 1970). We have only to look at the developmental genetic literature to convince ourselves that there are literally thousands of genes with allelic variants providing a vast potential source of developmental variation. Indeed, recent authors have correlated changes in genes in natural populations with phenotypic changes either within a population (Gibson and Hogness, 1996; Dworkin *et al.*, 2003) or between related taxa (Sucena *et al.*, 2003). As Müller and Newman (2003) note, the gene-centered neo-Darwinian paradigm has much to recommend it, but it may be insufficient for understanding the origins of the phenotype; the results of genome projects showing a largely conserved repertoire of genes in organisms of vastly different form should inspire us to seek additional factors for the origins of developmental variation. It is telling that T. H. Morgan's 1907 text *Experimental Zoology* had chapters entitled "The Influence of External Condition in Causing Changes in the Structure of Animals" and "The Inherited Effects of Changes Induced by External Forms." Within a few decades, such topics would not appear prominently in a reputable text. After nearly a century of neglect, there appears to be a growing willingness to explore alternatives to a mutational origin for developmental and evolutionary variation (see West-Eberhard, 2003, Chapter 26).

A potentially important source of developmental variation is variation resulting from the dynamics of developmental processes themselves. If this variation exists

but is not attributable to genetic change, then it might be a phenomenon of interest to developmental biologists, but can it bear on evolutionary change? The purpose of this chapter is to pose the question: Can developmental processes themselves generate variation that plays a role in evolutionary change? I call this potential source of variation "intrinsic developmental variation." I explore the question of whether or not intrinsic developmental variation exists and the conditions under which it might be revealed. For such variations to be a robust feature of evolutionary change, they must be "captured" genetically. In other words, the variation would have to precede the genetic changes, rather than being the result of them, as is usually assumed in current conceptions of the causal links in evolutionary change. If intrinsic developmental variation is an important factor in the evolution of development, then developmental biologists must devote more effort to understanding the origin of the "spontaneous" developmental variations we now ignore as impediments to doing controlled experiments.

I. DOES INTRINSIC DEVELOPMENTAL VARIATION EXIST?

Anecdotal evidence suggests that intrinsic variation in development is alive and well. The frustration expressed in the Harvard Law of Biology "under the most carefully controlled conditions, biological material does whatever it damn well pleases" is familiar to most of us working at the bench; we struggle to eliminate genetic and environmental factors, and yet development is variable.

To illustrate the different levels of organization in which developmental variation may arise, I describe a small sample of fruit fly variants that appear in uniform environments among individuals with the same genotype. No doubt, other developmental biologists will bring to mind additional examples.

At the molecular level, the distribution of gene products may vary from organism to organism. An example to which we will return is *bicoid* in flies. *bicoid* is a maternal effect gene with a DNA-binding motif whose message is deposited in the anterior portion of the egg. It binds to important anterior determining genes such as *hunchback* (*hb*). When *bicoid* is removed, the head region is posteriorized. The protein localization boundary can vary in different embryos and extend to as far as 30% of the embryo (Houschmandeh *et al.*, 2001).

Despite this inherent variation, adult morphology seems unimpaired by the "sloppiness" of the localization. At the level of cell organelle, the oocyte nucleus migrates through the cell to its final position in the future anterior of the embryo. The paths of the nuclei during migration vary in different embryos, but they all end up in the same region of the developing egg (Roth *et al.*, 1999). At the cellular level, division patterns are rarely predictable (development in the nematode *Caenorhabditis elegans* is an exception). Variable patterns of cell division can be seen by observing

mitotic patterns in bilateral structures. In several kinds of organisms, somatic recombination may be used to produce genetically marked mitotic clones during development that can be distinguished from other groups of cells by a convenient marker such as color. If one compares clone size and shape at a particular landmark—the anterior/posterior boundary in the wings of flies—they may vary in the wings of the same organism as well as between organisms. Nevertheless, the adult wings are usually bilaterally symmetric and have virtually identical shapes both within and between organisms.

Deviations from bilateral symmetry often provide evidence that intrinsic developmental variation is producing a phenotypic effect. For example, the work of Smith and Sondhi (1960) indicated that, in the *ocelliless* mutation in *Drosophila subobscura*, it was possible to select for loss of either left or right posterior ocellus, but it was not possible to specify which one. The authors suggested that genetic variation for asymmetry exists, but the direction of the asymmetry is not genetically specified. If so, we can conclude that intrinsic developmental variation may determine that only one ocellus will be present, but this will have little evolutionary impact because there is no genetic variation to fix the direction of the asymmetry. The explanations for these observations are as enigmatic as they are important. Lobster claws also show random left–right asymmetry, but this has its explanation in differential use (Govind and Pearce, 1989). If one claw is bound with an elastic band, the other claw will be the one to grow large.

Clearly, not all left–right asymmetries are indeterminate. Land slug contractile lung openings are found uniformly on one side of the head as a result of counterclockwise torsion (Brusca and Brusca, 1990), and narwhal males have a developed left tusk (but see Bateson [1894] for bilateral tusks and other anomalies of asymmetric species). Presumably there is genetic variation associated with the reliability of these asymmetries.

Curiously, one of the phenomena pointing most clearly in the direction of intrinsic developmental variation occurs when mutant strains arise. It is underappreciated that many mutations are not expressed to the same extent in all individuals, and in fact, a reproducible proportion of individuals in a stock homozygous for a mutant allele may appear to be wild type (variable penetrance); if wild-type-appearing parents are bred together, the characteristic proportion of mutant and wild phenotypes will be found in their progeny. Sometimes the frequency of mutant progeny can be raised or lowered in the stock by selection of mutant or wild-type phenotypes respectively, yet it is often difficult to achieve 100% penetrance. Indeed, when choosing mutant stocks for classroom purposes, we often select for higher penetrance and expressivity shortly before students receive them. One explanation for this variation is that the mutation produces a perturbation that the usual buffering mechanisms are unable to deal with, thus revealing intrinsic variation.

Often, mutations are buffered during selection for the wild-type phenotype, illustrating that genetic variation may exist for this buffering (here synonymous

with canalization). Variation in response to teratogens or phenocopy agents can be interpreted similarly as a failure of canalization in the face of perturbation. The same genotypes under the same environmental conditions produce variable phenotypes. The almost ubiquitous existence of canalizing genes, which can modulate perturbations of genetic or environmental origin, points to the possibility that variation from intrinsic causes can often be fixed (either in the mutant or wild-type form) by genetic "capture."

Those of us who deal frequently with variable penetrance and expressivity are driven to wonder what is special about those unusual genetic variations that can be relied on to have 100% expressivity and penetrance. One explanation is that they act in a part of a network in which regulation is difficult. According to this kind of thinking, the place in a network rather than the nature of the gene product is crucial. An anatomic analogy might give us some intuition here.

We are concerned about heart attacks but not hand attacks. A reason for this is that, in most regions of our bodies, arteries feeding tissues are anastomosed, e.g., arranged in a ladderlike pattern (Figure 7-1A). In contrast, coronary arteries lack such connections (Figure 7-1B); in consequence, blockage of a coronary artery leads to the death of cells fed by the artery below the blockage. Blockages elsewhere affect little tissue because blood can flow in from connecting vessels. The structure of the arterial network, not the nature of arteries or the blockage, renders the heart and brain vulnerable to vascular mishaps. Likewise, genetic mutations may have greater or lesser effects depending on the nature of the network they are part of, not on the quality of the mutational change.

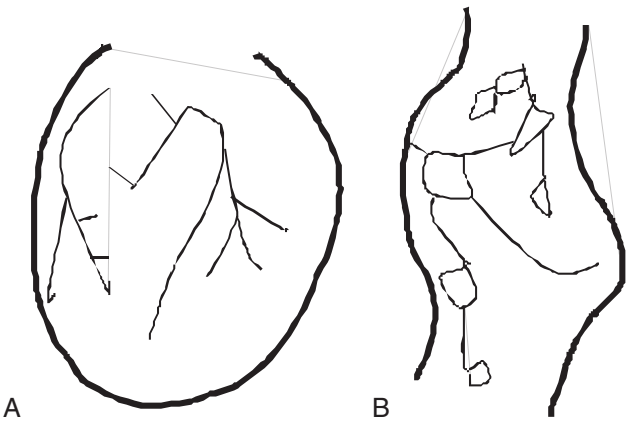

FIGURE 7-1. (A) Cartoon of nonanastomosing coronary arteries of the right heart ventricle and (B) of anastomosing arteries in the knee (after J. M. B. Grant, *The Atlas of Anatomy,* 1956).

II. INTRINSIC VARIATION IN DIFFERENT ENVIRONMENTS

Intrinsic developmental variation described so far has been phenotypic variation occurring under the same genotypic and environmental conditions. It was suggested that variation fitting this description is released when developing systems are perturbed and the usual buffering systems do not cope. This is a form of developmental plasticity and raises the question of the relationship of developmental plasticity to the evolution of development.

Developmental plasticity and its consequences have been reviewed at length in West-Eberhard (2003). I merely wish to point out here that a phenotype arising from a failure to buffer may be fixed genetically through selection, either as the sole phenotype in a variety of environments or as an alternative conditional phenotype, dependent on the environment (polyphenism) and thus contribute to evolution. This is the phenomenon of genetic assimilation (Waddington, 1953; Dworkin and Gibson, this book).

One may now ask, is there a difference between genetically modulated variation and intrinsic variation? Because organisms have an evolutionary history, intrinsic and genetic variations are intimately connected, and as stressed by Newman and Müller (2001), may be difficult to disentangle. It seems to me that there may be a difference between the two types of variation in that the appearance of intrinsic variation is biased by existing structures both material and abstract (regulatory networks) as well as by the physics of development. In contrast, mutationally derived variation is usually considered to be random (although Yampolsky and Stoltzfus [2001] discuss the possibility that mutationally derived variation is also biased). In any event, both the view in this chapter and the position of Yampolsky and Stoltzfus is that more effort should be devoted to "internalist" explanations for variation in evolution.

III. POTENTIAL ORIGINS OF INTRINSIC DEVELOPMENTAL VARIATION

A. Noise

The term *noise* is used in common parlance as well as in a technical sense. A feeling for noise in the technical sense can be gleaned from the following example.

Suppose we tracked the proliferation rate of individual blastomeres in a developing embryo. Each cell would have a particular rate that could be averaged to produce a population mean. The deviation of each cell rate from the mean of the population produces a measure of population variance. Suppose there was little noise in the system and all the cells divided virtually synchronously. In such a case, the population mean would mirror the individual means. Suppose, however,

that there are two groups of cells, one that divides at one frequency and the other that does not divide at all. In this scenario, the population mean does not reflect this bimodal population, and the variance is high. In most cases, phenotypic variability appears to be stochastic (e.g., random), and this property is formally called "noise." A measure of this is:

population variance in phenotype/population mean.

Noise can be measured during development at every level of biologic organization from molecule to adult phenotype. Can we determine the processes that give rise to noise? Does noise at one level or time of development give rise to noise at higher levels or later times? Is noise a necessary aspect of development? Can it contribute to developmental variation with evolutionary consequences?

IV. AN EXAMPLE OF NOISE IN EUKARYOTIC TRANSCRIPTION

Baker's yeast, *Saccharomyces cerevisiae*, is an excellent system in which to search for noise emerging from basic eukaryotic gene expression systems that might provide a source of developmental variation in multicellular development. This yeast is well characterized genetically, and experiments can be done on clonal derivatives, reducing the probability that observed variations are due to mutations. For example, Blake *et al.* (2003) constructed an ingenious system of transcriptional regulation in which the reporter gene, green fluorescent protein (GFP), was positively driven by the nutrient galactose as well as by an inhibitor (anhydrotetracycline) of a transcriptional inhibitor (tetracycline). GFP expression was monitored by measuring fluorescence on a per-cell basis in a flow cytometer. By varying galactose and anhydrotetracycline, noise was varied from low to high. Under low-noise conditions, cells showed a unimodal distribution of GFP production, whereas distinctly bimodal distributions were found under high-noise conditions. Thus cell populations with low or high GFP synthesis can emerge from a uniform population by increasing noise in the transcriptional system. Increasing translational efficiency in this system amplified the cell-to-cell variation stemming from noisy transcription. These results suggest that nongenetic sources of cell variation can lead to cell differentiation. In this case, a threshold response to GFP quantity could divert the quantitative differences between cells into qualitatively different cell differentiation.

Is noise, as measured by stochastic gene activation, a feature of normal development? In their review of enhancer action, Fiering *et al.* (2000) suggest that enhancers may increase the probability of transcription of a gene rather than its rate of transcription. They bring together evidence suggesting that enhancers are regulators of inherently stochastic transcription. Not only are there stochastic aspects of

transcription at the level of individual genes, it has long been held that inactivation of one X chromosome in each cell of female mammals is random (Lyon, 1961).

Is noise sufficiently reliable to generate predictable developmental trajectories? The work reviewed by Fiering *et al.*, described above, suggests that it is. Does noise always yield phenotypic consequences? The next example suggests that it may not.

V. NOISY *BICOID* GENE EXPRESSION IN FRUIT FLIES

It came as a surprise to many of us that one of the premier gradients for organizing early embryogenesis in flies, the bicoid (Bcd) protein gradient, was variable in terms of its posterior boundary (Houchmandzadeh *et al.*, 2002). The variation in the Bcd boundary location was on the order of five cell nuclei, whereas, surprisingly, one of the genes downstream of *bcd*, namely *hunchback (hb)*, had a precise protein boundary location with respect to embryo length with an error rate of less than one nucleus. Apparently, sloppiness of some gene expression, such as that of *bcd*, is tolerated in development and evolution if its consequences can be filtered out, in this case by *hb*. Although it is not understood how *hb* can be regulated by *bcd* as well as other genes to produce its precise expression pattern, this example shows that developmental variation at the level of gene expression may be more common than we might have thought and that such variation may have little developmental consequence.

VI. NOISE IN ASYMMETRY PRODUCTION

I have mentioned asymmetry as a form of developmental variation. The source of developmental and genetic variation resulting in asymmetry has been explored by Palmer *et al.* (1993). They consider subtle asymmetries, for example, fluctuating left–right asymmetry, presumably the result of randomness introduced by molecular movements, rates of physiologic processes, and cell division and growth. They also discuss macroscopic asymmetries such as the antisymmetry of lobster claws and directional asymmetry as found in the preponderance of human right-handedness. According to their analysis, the one factor common to the origin of all these asymmetries is "noise." For fluctuating asymmetry, this is the only developmental feature they associate with its origin.

VII. NOISY IMPLICATION FOR EVOLUTION

The lack of phenotypic consequence of some intrinsic developmental variation does not mean that such variation has no potential evolutionary consequence;

unless we are willing to argue that the enormous amount of hidden genetic variation revealed by twentieth-century population geneticists has no potential evolutionary significance either. Just as the effects of genetic variation may be suppressed by other genes, genes can also be selected to suppress or enhance intrinsic developmental variation as discussed above for enhancers. Put another way, genes can "capture" developmental variation or suppress it. Indeed, managing intrinsic developmental variation is an important function of canalization (see Chapters 9 and 21, this book). The possibility that genes "capture" forms produced by nongenetic processes and make them part of the genetically controlled morphologic repertoire was proposed by Newman (Newman, 1992, 2003; Newman and Müller, 2001) to have been important in the origin of biologic form. There is reason to suppose that variation arising from intrinsic developmental processes can be "captured" genetically. Experiments suggesting this have usually involved stressing organisms under conditions in which a small number of individuals show morphologic abnormalities. Continued selection of the abnormalities under stressful conditions may lead to the "assimilation" of the abnormal morphology even without stress (Waddington, 1952), and it demonstrates that genetic variation is often available to push developmental trajectories in new directions. The gene capture idea could well be tested in the yeast experimental system described above. Using flow cytometry, high and low GFP-producing lines could be established and continuously selected under conditions of high noise. A prediction of the gene capture scenario is that eventually, even in noisy conditions, the strains will no longer produce a bistable population of cells but rather either a high or low GFP-producing line depending on the selection regimen. Because the cells were originally clonal and the yeast is not undergoing meiosis, most genetic variation involved would be due to chromosomal rearrangements or mutation. In principle, the genetic changes involved could be determined because the yeast genome has been sequenced. So long as we are performing thought experiments, we should probably have several replicates of each selection line and ask if the genetic changes in each line are similar. Based on my experience with flies, I would guess that different genes for GFP production regulation would arise in the different lines.

VIII. NETWORKS

Our descriptions of development often involve the construction of "wiring diagrams" in which molecular pathways or even cellular and tissue inductive relationships are described. Such networks are an important abstraction in thinking about development because, fundamentally, development is about coordination, the coordination of cell behavior to make forms and the coordination of morphogenesis and both cell differentiation and patterns of differentiated cells. Networks provide a global way of describing unidirectional inductions and mutual

interactions important for coordination. These are useful in understanding how qualitative differences between cells or regions may arise, but of particular interest here are emergent properties of these networks that depend primarily on the pattern of connectivity and not on the quality of the types of molecules, cells, or tissues involved.

As discussed earlier, in connection with blood vessel continuity, network structure affects the viability of the system after perturbation (in this case, blockage). We will review suggestions that different patterns of connectivity may be associated with different levels of noise (stochastic behavior) and that hence patterns of connection may be a source of intrinsic variation, which may themselves evolve in ways that modify the developmental variation produced. There are a variety of vantage points from which to consider networks, and we will discuss a few of these. As with the general subject of noise, we are at the beginning of a period of exploration of network consequences for development and evolution that involves both theory and experiment. The knowledge base we have erected in terms of genomics as well as of developmental pathways coupled with our increasing toolbox of transgenic techniques to alter network morphology provides a basis for experimental tests of network behavior predicted from models and simulations.

The assertion that pattern of network connectivity (topology) itself may be an important aspect of development, and its variation has been inferred from theoretical work, simulations of theoretical systems, and simulations of abstracted "real life" networks.

My first exposure to the possibility that pathway structure itself could be important was the work of Kacser (1957), who suggested from thermodynamic principles that, in a linear metabolic chain of substrates, the output of product would be insensitive to most fluctuations in enzyme efficiencies but that the situation would change if there were a branch point (Figure 7-2). In this case, changing enzyme efficiency near the branch point would modify the rate of product production. This work was followed up by Kacser and Burns (1981) with a study of metabolic mutants in *Neurospora* in which the theoretical predictions were largely borne out.

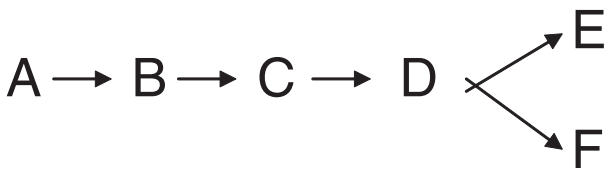

FIGURE 7-2. Pathway structure plays a role in pathway dynamics. If A–F are substrates and products in a pathway, mutations which reduce the rate at which Band C are made, will not affect the qualities of products E and F. If a mutation reduces the amount of D that is available, quantities of E and F produced will be affected.

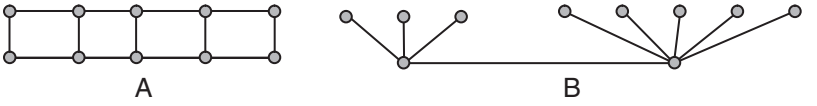

FIGURE 7-3. Two network configurations: (A) each node has two or three connections, (B) nodes have either two connections or three or more. In each case there are 10 nodes.

Another approach to biological networks was undertaken by Jeong *et al.* (2000) who explored the networks of core metabolic pathways in 43 organisms ranging from Archaea to eukaryotes and asked whether the connectivity observed consisted of substrate nodes that all had the same number of links or whether the topology comprised a few nodes (hubs) with a large number of links connecting nodes with fewer links to other nodes (Figure 7-3). The overwhelming conclusion was that the latter topology best describes metabolic networks. From what we know about signal transduction and transcription cascades in which a few pathways are used in a variety of different contexts (Hultmark, 1994; Wilkins, 2002), I would predict that the same will be found for these pathways in developmental contexts. Jeong *et al.* (2000) speculated that this pattern of connectedness is indicative of selection for "robust and error-tolerant networks."

Approaches to examining more developmental networks have been pursued with emphasis on well-studied pathways such as the segment polarity gene network in fruit flies. Starting with known relationships between segment polarity genes, Von Dassow *et al.* (2000) constructed a model of the network using differential equations, which included parameters such as protein and messenger RNA half-lives as well as binding rates and cooperativity coefficients. The authors then performed simulations using different parameter values and initial conditions to see what values mimicked the behavior of the system being modeled. To their surprise, a wide range of initial conditions and parameter values were capable of doing this, and they surmised that this robust behavior results from the topology of the network. These two examples suggest to me that contemporary networks have been selected for robustness and that probing their potential for producing developmental variation requires finding special cases in which noise is "allowed" to exert a phenotypic effect. The stochastic nature of transcriptional initiation might be such an opportunity as would a new environment in which the networks have not been selected.

The question of how the structure of developmental networks might evolve has been explored by Salazar-Ciudad *et al.* (2001a,b). They constructed a model involving a string of nuclei each with three interrelated genes, paracrine factors that can act on genes with activating or inhibiting consequences, and dynamic equations that they obey. To simulate evolution, they started with a 100 arrays of nuclei strings, ran

the simulation, and looked for patterns in which nuclei differed in the production of a given gene product. They selected for the next generation of simulations the 50 arrays with a pattern closest to a preselected stripe pattern. After 100 simulations, they examined the relationships between the genes, the paracrine factors, and the number of stripes they produced. Two types of networks could be discerned, those in which components had mutual interactions (called emergent networks) and hierarchic networks in which the influence of the network elements flowed only in one direction. Further comparisons suggested that emergent networks produced more complex but also more variable patterns than did the hierarchic networks and for the same degree of pattern complexity (in their case, the number of stripes). They were simpler at a molecular level, requiring fewer genes. Some tantalizing speculations arise from these findings. We might expect that emergent networks could generate morphologic innovations and then be replaced by more robust hierarchic networks in evolution. On the other hand, traits showing rapid change in response to varying environments might be based on more plastic, emergent networks.

This selective sojourn into biological networks and their potential for generating developmental variation suggests that network structure has the potential to influence the extent and nature of developmental variation. Exploring network topology in developing systems can be facilitated by our developing technologic capabilities both with computational strategies that can infer network relationships from genome databases (Jeong *et al.*, 2001) or from RNA expression changes (Gardner *et al.*, 2003). A difficult challenge is to determine whether intrinsic developmental variations arising from networks/noise have contributed in the past and are contributing presently to the evolution of development. If these intrinsic sources of variation are, or have been, important, we must alter our worldview of evolution as being driven by mutation. In this scenario, mutation is only one force in evolution; intrinsic variation that produces advantageous phenotypes could be an additional factor if the phenotypes are "captured" by portions of standing genetic variation that reinforces the adaptive phenotype. This is not a new idea. As mentioned, it is in keeping with Newman's explanation of production of multicellular forms (2003) and with West-Eberhard's suggestion (2003) that developmental plasticity may first produce phenotypes that may then be honed and fixed by genetic variation. This question remains: Is the origin of developmental variation always "genes first," or is there frequently a source of intrinsic developmental variation producing a phenotype that is at first only indirectly dependent on genotype and then "captured" genetically, as a result of selection? The idea that phenotypes may arise before the alleles that stabilize them has never been easy to accept and accounts for the reluctance to accept the work of Baldwin (1896), who proposed that learned capabilities might have evolutionary consequences. Because "development first" is a substantial modification to our received wisdom about evolution, it is of sufficient importance to explore the possibility in greater depth.

IX. MORPHOGENETIC FIELDS: A POTENTIAL SOURCE OF VARIATION

The concept of the morphogenetic field as a unit (module) of development has enthralled and appalled generations of developmental biologists. Morphogenetic fields are defined operationally as those cells in a developing organism that, when transplanted elsewhere, will form a particular structure, say, a wing. The potential wing-forming region of a chick is greater than the region that usually produces the wing. When transplanted, this latter region produces a wing more frequently. The propensity to form a wing decreases in a graded fashion, hence the term *field* as analogous to physical fields. Biologists were enthralled because so many structures arise from primordia that express field properties (gradient of strength, ability to duplicate or regenerate) with self-organizing emergent features and appalled because they do not understand how these general properties arise, although sometimes particular gene expression can be correlated with the ability of cells to participate in a field (Technau *et al.*, 2000; Gilbert *et al.*, 1996).

My interest here is that morphogenetic fields are a potential locus for stimulating phenotypic change (an excitable medium in the terminology of some). Dramatic examples of malformations attributable to field responses are cyclopia in vertebrates caused by the failure of the normal "emancipation" of two eye fields from an initial eye primordium and extra frog legs, probably induced by parasites stimulating mirror image duplications in leg fields. We assume that morphogenetic field properties depend on cell–cell communication because surgical bifurcation can stimulate the production of two fields from one. Morphogenetic fields may be a reasonable place to look for intrinsic developmental disturbances because changes in cell behaviors, however triggered, may lead to modifications in the phenotype produced by the field. For example, enlargement of a field by excess cell proliferation or cell size might impair communication and lead to field duplications just as field bifurcation does (Buratovich and Bryant, 1995, 1997). Thus modifying the noise or the network organization of fields might provide a useful means to explore effects of intrinsic developmental variation. Because fields are inherently self-organizing and regulating for example, a partial field is able to develop a whole structure, there will be a tendency for field changes to be coordinated. The ability to induce eyes on antennae, legs, and wings by ectopically expressing the *eyeless* gene in different imaginal discs of fruit flies is a testament to the potential of perturbed fields to produce large phenotypic changes. Fields are potentially influenced by internal processes and genes or by external physical, chemical, or biologic (for example infection) perturbations. Genetic and environmental influences on *obake*, a mutation causing duplications of antenna and other head fields, were studied by Atallah *et al.* (2004). They show that specific mutations,

natural genetic variation, and larval density affect the mean number of antenna duplications. Furthermore, the duplicating ability of fields offers the possibility of new morphologies, much as gene duplication has long been considered a source of gene family evolution. The formation of a biramous insect appendage in the uniramous insect taxon (Dworkin *et al.*, 2001) depended on the duplication of the antenna field. No doubt the Greeks had the power of morphogenetic fields in mind when they described Pegasus, a four-legged animal with additional wings.

X. IMPLICATIONS

One of the implications of a "development first" view of the origin of developmental variation affects the way we think of "mechanisms of development." In our current thinking, mechanisms revolve around the genes that, if altered, modify the developmental outcome. It is then natural to think of the genes as primary not only in evolution but in mechanisms of development as well. If asked how a limb develops, we first tend to recall signal transduction and transcription cascades rather than the cells and self-organizing fields in which these cascades play a role. If we find evidence that genes may also capture morphologies (analogous to turning an oral tradition into a written record), we will turn our attention from the words to the underlying story; words only have meaning in the context of a particular language at a particular time in history, and different words can tell the same story. Genes and genetic cascades are used for different purposes within an organism and in different taxa. It is as if we observe development like aliens coming to earth who try to understand the marvels of electric lights, stereos, televisions, computers, and all the other devices they see, by studying wall switches and fuse boxes. If we focus on intrinsic variation and its genetic capture, we will see development less as a canonical series of events and more as a dynamic where there is no single way of making a limb, but a dynamically changing group of ways with some similarities and some differences depending on context (rest of the genotype and environment). Sometimes these differences will produce the same phenotypes as other ways and sometimes different phenotypes.

From an evolutionary perspective, restrictions would be reduced if intrinsic developmental variation were an important source of evolutionary variation. For intrinsic developmental variation to have a plausible role in evolution, it must undergo "genetic capture." As suggested in this chapter, this might happen if an adaptive intrinsic developmental variant fortuitously contained alleles or allele combinations promoting that phenotype even at a level below a phenotypic expression threshold. In this way, the fortuitous genotype would increase in the population. The recurring nature of the developmental variant (because the structure of the developing system gives rise to a biased set of variants) would allow time for

accumulation of additional alleles that in sum produce the phenotype "on their own." I am grateful to M. J. West-Eberhard for pointing out that Sewall Wright's ideas on random genetic drift in subpopulations could provide a genetic basis for this scenario. According to Wright's analysis (Wright, 1948), subpopulations can become genetically differentiated from one another because of random genetic drift, even in the absence of selection. As envisioned here, drift coupled with adaptive developmental variants could produce genetically based (adaptive) phenotypes more rapidly than if drift were operating on its own.

Even if plausible, is this a common mechanism for evolutionary change? It is usually impossible to recover the history of a change based on its present state, and I do not see a way of detecting a "signature" of developmental variants that have become genetically assimilated. Instead, I believe we must turn to the kind of laboratory experiments I suggested previously and hope that insights gained will allow us to explore the natural world more perceptively.

In his provocative book *Internal Factors in Evolution,* Whyte (1965) suggested that, in the history of physics, a nineteenth-century preoccupation with randomness in the universe gave way to an interest in order and structure in the twentieth. I suggest that, in twenty-first-century developmental biology, we combine the issues of order and randomness by investigating the effect of internal noise on the behavior of ordered structures to gain a more realistic view of the origin of developmental variation.

XI. SUMMARY

1. In considering the origin of developmental variation, we must consider the contribution of both genetic (mutational) sources and sources of variation arising from developmental processes themselves (intrinsic sources of developmental variation).
2. Phenotypic effects of intrinsic developmental variation are seen in phenomena ranging from fluctuating asymmetry to variable penetrance and expressivity of alleles.
3. Two sources of developmental variation are noise and perturbation of network structure.
4. From evolutionary perspectives, it is possible to conceive that variation arose first as intrinsic variation and then was captured genetically by abundant genetic variation.
5. Such a scenario provides an alternative to the "genes first" ideas of neo-Darwinism and provides additional avenues for rapid and saltational changes in the evolution of development as well as for gradual change.

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