Origination of Organismal Form

Beyond the Gene in Developmental and Evolutionary Biology.

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14 Phenotypic Plasticity and Evolution by Genetic Assimilation

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Evolution by natural selection occurs whenever the following three conditions hold: phenotypic variation, differential reproductive success, and heritability. The last of these conditions is important because, in the absence of heritability, there is no (additive) genetic component to the overall phenotypic variance. At the same time, one knows that a given phenotype develops only in the context of a specified environment (see Gilbert, chapter 6, this volume). Moreover, the same genotype can result in different phenotypes in different environments, thereby defining a reaction norm (Suzuki et al., 1986). The concept of reaction norm is related to that of phenotypic plasticity, the capacity of development to lead to the appropriate (i.e., adaptive) phenotype in the appropriate the environment provided that environment falls within the range of experience of the organism's ancestors.

This chapter explores a different aspect of phenotypic plasticity, namely the ability of a genotype to give rise to more than one phenotype even in the same environment. This means that whether the environment is uniform or not, the relationship between genotype and phenotype is one to many and not one to one. We note in passing that multicellular development is a familiar example of cells with the same genome exhibiting extremely diverse phenotypes. However, excepting organisms in which early embryonic development is highly regulative (e.g., mammals), the internal environment in the unfertilized egg is often functionally heterogeneous (Slack, 1985). The implication is that the diversity of cellular phenotypes must just as often be traceable to environmental influences, in this case an environment of maternal origin.

The biological differences between individuals can come about both because of differences in their genetic makeup and because of *epigenetic* factors. The latter include physical factors, secondary modifications in DNA sequence or structure, and variations in patterns of gene expression. Epigenetic factors lie behind the obvious differences between cells of one tissue type and another in an organism. Two individuals that are genetically identical (or virtually so), such as monozygotic twins, can differ in their phenotypes, and therefore differ epigenetically. The differences can be largely or entirely environmental in origin. For example, they can be caused by differences in early postnatal sensory experiences (Hubel, 1988). But often, because we cannot readily ascribe the differences to the environment, we put them down to "developmental noise," a form of random, uncontrolled variation between individuals (Waddington, 1957).

Partly on account of its name, developmental noise tends to be regarded as an unfortunate, but unavoidable, lack of precision in developmental systems, in short, as a nuisance. To the contrary, I argue that developmental noise is yet another manifestation of the organism's being "plastic" in its extended sense of "capable of adapting to varying conditions"

(Merriam-Webster, 1993, 890). The term *phenotypic plasticity* accommodates the possibility that developmental noise can also originate from environmental causes. Besides the external world, "environment" can include the internal microworld of cells and organisms. Indeed, variations that occur independently of spatial or temporal heterogeneities in the external environment can be exploited for the purpose of evolutionary change. As I hope to show in this chapter, given the right circumstances, phenotypic plasticity can play an important role in evolutionary change. (Figure 14.1 summarizes the essence of my argument.) My use of "plasticity" does not demand that the new phenotype be adaptive, although it is hardly necessary to add that grossly maladaptive phenotypic changes are unlikely to survive in the long run.

Many of these ideas, in particular the notion that developmental noise or phenotypic plasticity could be a significant factor in evolutionary change, were anticipated by Bonner

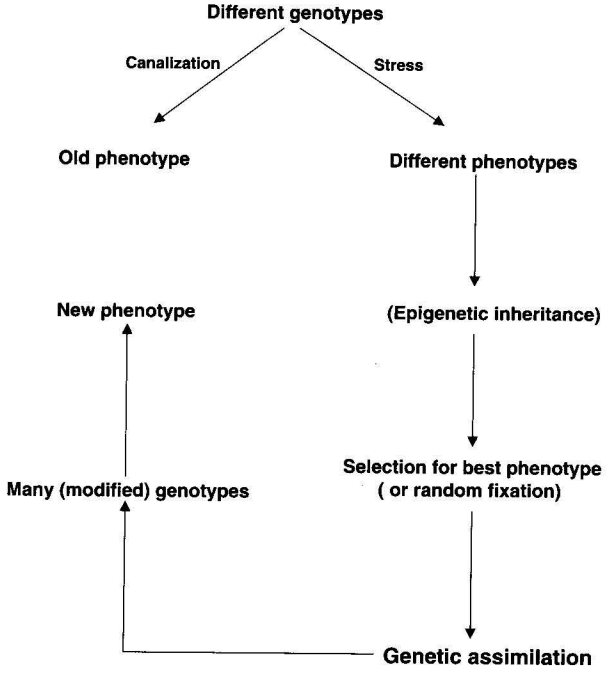

Figure 14.1
Flowchart indicating how genetic assimilation might lead to rapid evolutionary change. A set of genotypes constitutes the "wild type" by virtue of being canalized for an optimal (old) phenotype in a certain environment. Because of genetic or environmental stress, canalization breaks down. This leads to the appearance of a spectrum of phenotypes; selection for the fittest phenotype is followed by its genetic assimilation and canalization. Epigenetic inheritance may be an additional, though not an essential, factor in maintaining the favored phenotype, whose development may also be supported by random fixation of alleles (not discussed in the text).

(1965, 1967) in terms of what he called range variation. One can do no better than quote in detail: ". . . what apparently is inherited is the ability to vary within certain limits. The variation is therefore not genetic variation in the conventional sense, for the range of variation is genetically determined but the size of any individual within that range is not" (Bonner, 1965, p. 95) [*Note:* "position" or "status" would be less restrictive than "size"]. What advantage might be served in having individual variants that are not genetically determined, in having "this unconventional and relatively haphazard form of inheritance where genetically identical individuals can differ phenotypically over a considerable spectrum"? (Bonner, 1965, p. 95). "The answer is clear"; in the specific case of the cellular slime mold *Dictyostelium discoideum* (of which more below), "if this were not the case it would be immediately impossible to use the variation for the kind of [evolutionarily stable] organizational hierarchy that we are suggesting here" (Bonner, 1965, p. 99). Bonner goes on to discuss the implications of range variation extensively, and the only aspect of his discussion about which one might quibble today is the group-selectionist viewpoint—which may not be wrong in the context of clonal populations of slime molds or ciliates anyway.

The chapter begins by listing examples of phenotypic variation between individuals of the same genotype in the same (uniform) external environment. After outlining plausible means for the origin of such variation, it describes a typical laboratory experiment carried out by Waddington (and repeated by many others), in which he showed that artificial selection, acting on phenotypic variations, can lead to a major morphological change within a very few generations. It provides a conceptual framework for modeling that outcome and speculates on how genetic assimilation may bear on evolutionary changes in nature.

The Same Genotype Can Give Rise to Different Phenotypes in the Same Environment

Ranging over many phyla or divisions, instances of significant phenotypic variation between individuals raised in the same environment abound. In some cases, the individuals are genotypically identical, such as members of a clone. In others, the observed variation clearly appears to be independent of any genotypic differences that might exist.

Instance 1: Bacteria

Spudich and Koshland (1976) demonstrated that clonally related *Escherichia* coli bacteria exhibit substantial differences in their chemotactic behavior under identical experimental conditions. Upon exposure to an attractant, the swimming behavior of cells changes transiently, with the time required to return to the prestimulus pattern of behavior (the response time) varying enormously from cell to cell. The outcome is a cell-to-cell behavioral flexibility that could conceivably prove advantageous to one clone over another. The

flexibility is almost certainly not genetic in origin. Although one might object that the external environment may not be exactly the same for all bacteria swimming in a liquid medium, a careful study of the experimental conditions suggests that this is unlikely. A more plausible hypothesis is that the bacteria exhibit different phenotypes on account of statistical fluctuations—"noise"—inherent in the internal biochemistry underlying their chemotactic behavior. This is especially likely if the relevant biochemistry includes autocatalytic reactions (Delbrück, 1940). If the capacity to exhibit such a high degree of phenotypic variation has a genetic basis, clones may possess heritable differences in the extent to which they display a spread in response times. Consequently, the trait may be subject to evolution by natural selection, with selection occurring between clones.

Instance 2: Protozoa

When starved after being raised in a common nutritive environment, genetically identical social amoebas of the species *Dictyostelium discoideum* begin to exhibit striking differences in cell-to-cell properties, differences that culminate in the death of some amoebas and the differentiation of the rest into spores. Nanjundiah and Bhogle (1995) have shown that at least formally, the differences can be ascribed to a stochastic or "coin-tossing" process in which cells acquire different predispositions with different probabilities. Although the predispositions cannot be heritable (because some cells die), one can show that they are correlated with differences that exist in the spore population of the preceding generation, for example, differences in autofluorescence and in the ability to take up certain dyes (Baskar, 1996). The point is that phenotypic differences between genetically identical amoebas, though not environmentally based, are important for the development of *D. discoideum* and may have played a role in the evolution of social behavior in the species.

Instance 3: Vertebrata

Ashoub and colleagues (1958) found that the temperature at which isogenic mice are raised crucially influences their weight at different development stages up to the age of four weeks. In general, the variability of mice raised at an extreme temperature (e.g., 5°C or 28°C) greatly exceeds the variability seen when they are raised at 21°C. Moreover, large litters tend to show greater interindividual variability in birth weights than small litters do. Here again is an instance of significant interindividual variation within a common, though not necessarily identical, genotype.

Thus phenotypic variability can be exhibited in a manner that is, first, epigenetic in origin and, second, made manifest between genetically identical (or very similar) individuals raised in a common environment. What evidence is there that the variations are heritable? In the specific instances just discussed, none. However, there are observations testifying to the fact that interindividual epigenetic differences can indeed be inherited.

Epigenetic Inheritance

The evidence concerning the inheritance of epigenetic traits is extensive, as are discussions of the possible evolutionary implications (Jablonka and Lamb, 1995). McLaren (1962) cites the case of babies born at high altitudes in Peru. These babies have heart defects at birth, presumably on account of anoxia. The fetuses born to mothers with heart defects also tend to have heart defects themselves, showing that the effect can extend over generations. Better-studied examples hint at possible mechanisms. Genetically identical bacteria growing in the same environment can differ in being able to metabolize lactose or not, and can faithfully pass down either characteristic to their progeny (Novick and Wiener, 1957). When seeds of flax, obtained from self-fertilized plants grown in diverse nutrient regimes, are compared in the same environment, subsequent generations show stably altered characteristics. The differences pertain to shape, developmental patterns, height, weight, and nuclear DNA content; the fundamental change appears to occur in DNA sequences coding for the 25S, 18S, and 5S ribosomal RNA (Cullis, 1988). In Mongolian gerbils, intrauterine hormonal interactions between pups in the same litter affect both adult morphology and behavior. Female fetuses that happen to develop between two male fetuses are exposed to high levels of testosterone, an androgenizing agent. Such females exhibit delayed puberty and low lifetime fecundity in comparison with females that, as fetuses, were situated between two other females. Androgenized females produce litters with significantly male-biased sex ratios. In consequence, they tend to have daughters that are themselves androgenized (Clark, Karpluk, and Galef, 1993). Nuclear transplantation experiments in the mouse show altered patterns of gene expression, in particular with respect to genes that encode an olfactory marker protein and components of urinary proteins (Roemer et al., 1997). These patterns persist in subsequent generations. Here the epigenetic change is correlated with changes in the level of DNA methylation in the relevant genes and is stably propagated through heredity.

What principles might underlie epigenetic inheritance? A genetic scheme due to Bussey and Fields (1974) accounts for the inheritance of alternative epigenetic states (figure 14.2). Their model is patterned on the mutual repression exhibited by the *cI* and *cro* genes in bacteriophage lambda. It depends on a network of positive and negative feedbacks between three genes, a regulatory gene (*R*) and two structural genes (*G1* and *G2*). The expression of *G1* by itself characterizes one phenotype and that of *G2* by itself characterizes a different phenotype. The R product represses *G1* and *G2* but activates *R*; the G1 product activates *G1* and inhibits *G2* and *R*; similarly, the *G2* product activates *G2* and inhibits *G1* and *R*. To begin with, let us say that the genes encoding *G1* and *G2* are repressed. An environmental influence (the inducer) modifies the R product in such a manner that it can no longer regulate its own production or that of G1. Then G1 will continue to be made in the absence of the R product.

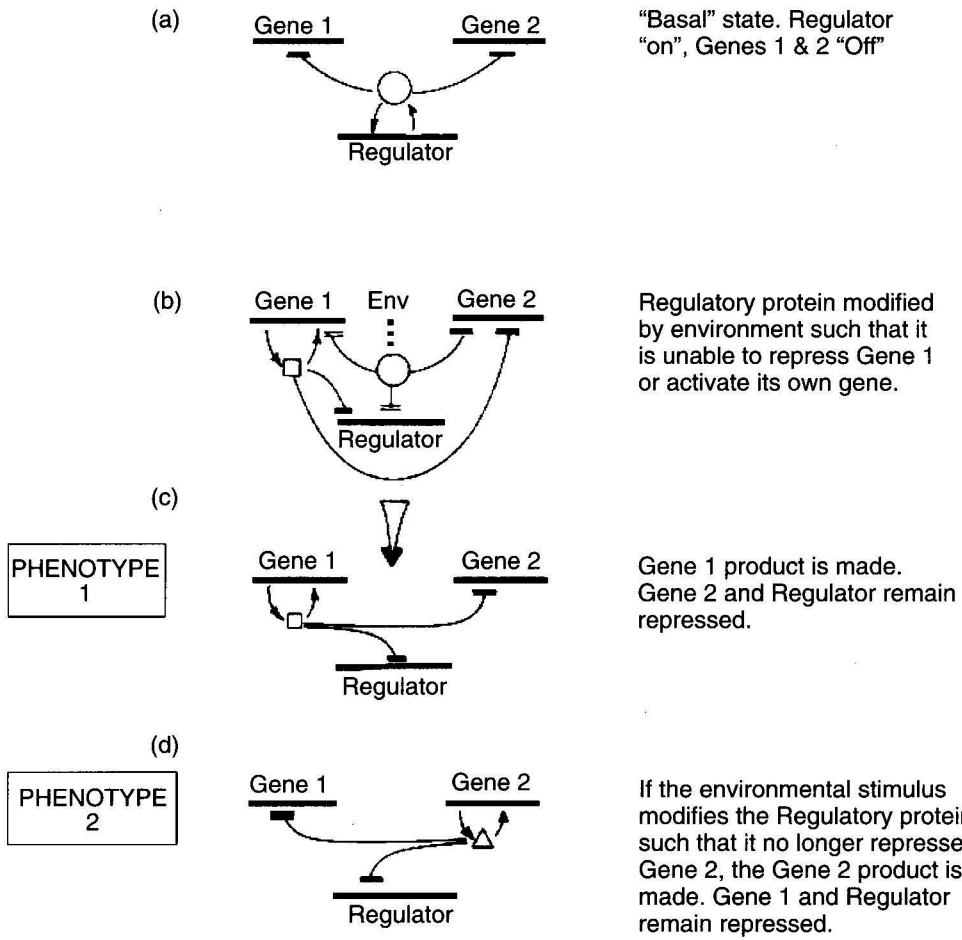

Figure 14.2
Model for hereditary transmission of epigenetically determined phenotypes. (After Bussey and Fields, 1974.)

A parallel series of steps ensures that the production of G2 is triggered and thereafter stably propagated. The explanation is similar to the one alluded to earlier that accounts for the stable transmission of the induced and uninduced states of the *lac* operon in *Escherichia coli* under specified conditions (Novick and Wiener, 1957). Namely, the medium in which the bacteria are grown is a "maintenance" medium. It has just sufficient lactose to ensure that cells with *lac* in an induced state have daughter cells in which *lac* continues to be induced, but not sufficient to cause induction in uninduced cells (or in their daughter cells).

We have seen that alternative phenotypes can be found in the same environment and can be propagated stably. Can such alternatives lead to interesting consequences? Yes, if there are many alternatives and their fitnesses are not the same. The argument rests on two facts. First, a stressful environment (one that departs sufficiently from the one to which the organism is adapted) can trigger phenotypic variation. Second, natural selection acts both on the mean value of a trait and on the extent to which there are variations about the mean. This puts a premium on the degree of reliability with which the optimal phenotype can be specified.

Phenotypic Differences Can Come About as a Result of Stress

Natural selection, in addition to molding the mean value of a trait, tends to improve the extent to which the trait is buffered. In other words, natural selection also sharpens the degree of precision with which the selected phenotype develops. Equivalent terms for *buffering* are *epigenetic stability* and *canalization* (Waddington, 1960). Here we consider the reverse, a breakdown of canalization and why the specification of the phenotype may become imprecise. The stress can be genetic or environmental in origin.

Genetic stress may be an inevitable correlate of developmental complexity. This can be inferred from the observation that organisms have evolved means for overcoming certain unavoidable consequences of possessing a complex internal biochemistry. The best-known example comes from studies of metabolic pathways made up of enzyme-catalyzed reactions, where the flux through a pathway constitutes an important component of the overall organismal phenotype. Kacser and Burns (1981) proved that when the pathway has a large number of intermediates, substrate concentrations are low, and the effects of feedback and nonlinearity are negligible, the flux is automatically buffered with respect to genetic stress. They showed that even when the level of an enzyme decreased by half (as might be caused when an individual was heterozygous for the wild type and a loss-of-function allele), the flux through the pathway changed to a negligible extent. The implication drawn by them was that, notwithstanding a wide range of possible stresses caused by changes in enzyme levels (which in turn could be due to mutations), metabolic fluxes are buffered—apparently automatically.

The assumptions made by Kacser and Burns, specifically, that substrate concentrations be low, feedbacks and nonlinearities unimportant, and oscillatory reactions not possible, turn out to be crucial. If one or more of these assumptions is violated, the system is no longer guaranteed to be insensitive to the effects of mutation, in particular the mutation of regulatory genes (Cornish-Bowden, 1987; Grossniklaus, Madhusudhan, and Nanjundiah, 1996). The resulting genetic stress can impinge significantly on the flux. Therefore, if the observation is that the phenotype, and so fitness, remains unaffected even under stressful

conditions, one might reasonably conclude that buffering mechanisms must have been selected for during the course of the organism's evolutionary history. Rutherford and Lindquist (1998) have provided a striking demonstration of how mutations in regulatory genes can destabilize development, conceivably by affecting the flux through one or more biochemical pathways. They found that when mutated, the *Hsp83* gene, which encodes the Hsp90 protein of *Drosophila melanogaster,* causes an enormous range of phenotypes to be manifested. (They went on to demonstrate that it was thereby possible to select for a novel phenotype; the mutant allele could even be dispensed with during the course of the selection.)

When organisms encounter abnormal situations, there may be no escaping the ensuing genetic stress. In normally outbred populations, inbreeding can cause genetic stress. In organisms that are normally inbreeders, such as flax, the *outcrossing* of two inbred strains imposes genetic stress. Inbreeding can lead to phenotypic variation in the progeny that goes well beyond the range found in outbred individuals. A widely accepted belief is that developmental pathways get destabilized by the high levels of homozygosity that follow from inbreeding. Why inbreeding has this effect remains unknown, but an observation made by Biémont, Aouar, and Arnault (1987) may offer a clue. These workers found that after sixty-nine generations of sib mating, an inbred line of *D. melanogaster* showed extensive reshuffling of the mobile genetic element *copia.* It appeared that a specific destabilization of the *copia* element had taken place on account of inbreeding.

The example of *copia* may point to a widespread source of genetic stress. The presence of mobile genetic elements, parasitic entities potentially harmful to their hosts, represents a trade-off between selection acting on the element to favor transposition and selection on the host to favor suppression of transposition. The result can be a stable polymorphism with respect to the distribution of the number of such mobile elements in the genomes of different individuals (Nanjundiah, 1985). Additionally, selection can act on the host so as to suppress transposition and thus improve the stability of its phenotype. But the transposition of mobile genetic elements is commonly replicative (Lewin, 1995) and involves obligatory events that are part of the physiology of the cell, such as transcription, DNA synthesis, and DNA recombination. Therefore, in attempting to suppress the replication of the parasite, the cell risks deleterious side effects that might result from interference with its own functioning.

A way to guard against such side effects might be to make an inhibitor of transposition in just sufficient amounts: one active copy of the gene encoding the inhibitor could block excessive spread of the foreign element, an optimal outcome from the point of view of the host. Two active copies might cause nonspecific deleterious effects; no copies at all would be useless of course. Inbreeding would disrupt this pattern of peaceful coexistence: some inbred lines would lack the inhibitor entirely, whereas others would suffer from a general depression of physiology; in short, the outcome would be dysgenic.

What about the destabilization caused by the *outcrossing* of highly inbred lines, something actually observed when the normal mode of reproduction involves inbreeding? The point to remember is that, irrespective of the nature of the accommodation that has been reached between the transposable DNA parasite and its host, it is unlikely that the same transposable element will have colonized all host lines. When two inbred lines are crossed, because both cellular and chromosomal environments are new for each set of elements, the resident parasitic elements in each genome may be released from the transposition block that existed in their normal hosts. Once again, the outcome can be expected to be dysgenic (Nanjundiah, 1985).

Environmental variations in space, time, or both can constitute an important source of phenotypic variation. Given a sufficiently unpredictable environment, there may simply be no single optimal phenotype (Levins, 1968). Even though environmental *variations* do not occur or are insignificant in the situations we are considering, the environment is stressful all the same. Under such conditions, stress can be a potent factor in eliciting large-scale phenotypic changes (Parsons, 1997). Heat shock, a good example of stress, can cause many cellular proteins to become dysfunctional (on account of abnormal folding, for example), thereby inducing other forms of damage to the cell. The cell tries to protect itself by increasing the rate of production of specialized stress proteins (formerly called "heat shock proteins") to sequester and dispose of the damaged proteins.

Many stress proteins are multifunctional. For example, Hsp90 plays a role in signal transduction, progress through the cell cycle, production of nitric oxide, transcription, and translation (Mayer and Bukau, 1999; Nathan, Vos, and Lindquist, 1997). In order to be able to carry out all these functions under normal conditions, it is evident that the cell must manufacture the stress proteins in sufficient amounts. When it mobilizes them for emergency functions, however, the cell could be compromising one or more of the normal functions mediated by stress proteins. If so, the effect would be a destabilized phenotype (Forsdyke, 1994). A stress-induced depletion of other regulatory molecules could be yet another route to destabilization. The outcome could be that crucial steps in cellular metabolism or gene activation are switched from "on" to "off" or vice versa. It is worth noting that genetic stress can also be thought of as a form of environmental stress, but this time with reference to the internal environment of the cell or organism.

Rapid and Heritable Phenotypic Change Can Occur by Means of Genetic Assimilation

Both Waddington (1953) and, independently, Schmalhausen (see Gilbert, 1994) predicted that if organisms varied genetically in terms of their ability to respond to an environmental stimulus by giving rise to novel phenotypes, natural selection could take advantage of that

ability. Their reasoning was based on the assumption that among the phenotypes that resulted as a consequence of the stimulus, some would have a higher reproductive fitness than others. Selection would enrich the population with genotypes that responded to the environment by developing the most advantageous phenotype in a reliable manner. In particular, selection would tend to increase the sensitivity of genotypes to the environment. Those genotypes would be most favored whose threshold of response to the stimulus was extremely low; indeed, so low that the response—the favored phenotype—continued to be expressed in the absence of the stimulus (Waddington, 1953). In this manner, a character originally acquired through exposure to a particular environment would now be stably inherited, or, as Waddington put it, "genetically assimilated."

Waddington went on to demonstrate that genetic assimilation could also work under conditions of artificial selection. Among the experiments carried out by him and repeated by others later, one set involved the transformation of a normal, two-winged stock of *Drosophila melanogaster* into flies with the four wings typical of the extreme Ultrabithorax phenotype (see figure 14.3; Waddington, 1956a,b; Ho et al., 1983; Gibson and Hogness, 1996). Two features made the process especially intriguing. First, it appeared impossible that mutational change could have taken place. Second, the number of generations over which selection needed to be practiced was quite small, of the order of 10–20. Thus genetic assimilation appeared—misleadingly, as Waddington was the first to point out—to resemble an evolutionary transition with Lamarckian overtones. Nevertheless, in his words, "if such a change occurred during phylogenesis it would certainly be accounted a macro-evolutionary phenomenon" (Waddington, 1956b, p. 1).

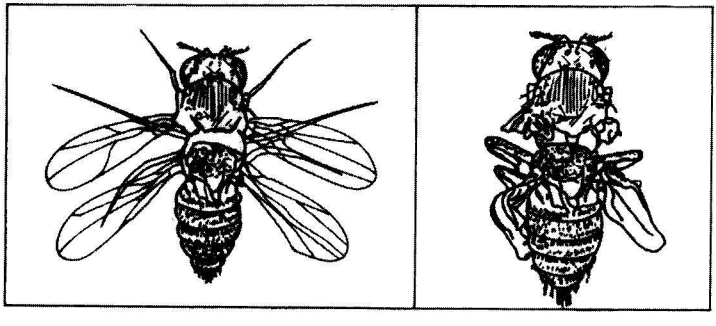

Figure 14.3
Schematic drawings based on originals. (*Left*) Multiply mutant fly with four wings. The anterior pair of wings is usual; the posterior pair is supernumerary and occurs because the tiny dorsal metathoracic appendages, the halteres, have been transformed into almost normal-looking wings. (After Suzuki et al., 1986.) (*Right*) Fly showing effects of genetic assimilation for the Ultrabithorax phenotype. The normal wings have been removed to show more clearly the modified, winglike halteres (dorsal metathorax → dorsal mesothorax transformation). (After Waddington, 1956b.)

The demonstration of genetic assimilation of the Ultrabithorax phenotype went roughly as follows. Eggs aged 2.5–3.5 hours were exposed to ether vapor for about 25 minutes. Some of the resulting adults resembled (not always very closely to begin with) four-winged flies characteristic of the *Ultrabithorax* genotype. Selection could be practiced by breeding from them: after many generations of selection these flies bred true for the new phenotype. Other demonstrations of genetic assimilation were more dramatic (Waddington, 1961), involving sib selection: breeding from the affected flies' brothers and sisters that had not themselves been exposed to the environmental stimulus. In such cases, neither the flies belonging to the assimilated stock nor any of their direct ancestors had ever experienced the stimulus.

In explaining genetic assimilation, Waddington made two assumptions: first, that canalization of the phenotype would have masked the existence of genetic variation in the original wild-type stock; and second, that new combinations of regulatory genes would arise in each generation through recombination. The environmental stimulus would merely unmask, so to speak, the underlying genetic variation (figure 14.4). In the experiment described above, Waddington managed to show by mapping that the selection regime had

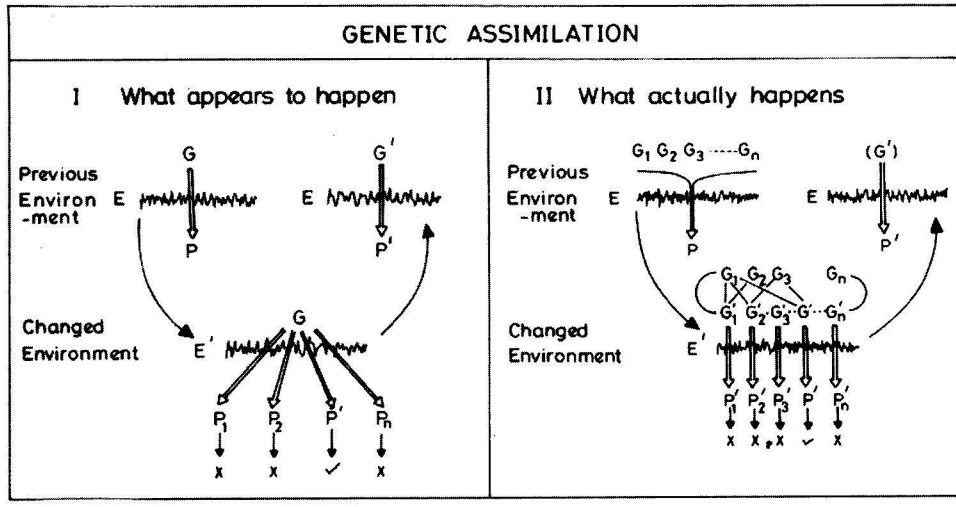

Figure 14.4
Genetic assimilation: appearance versus reality. Panel I assumes a one-to-one relationship between genotype **G** and phenotype **P** in the normal environment **E**, altered to a one-to-many relationship in the stressful environment **E′**. Selection for phenotype **P′** followed by a return to the previous environment results in a new phenotype that breeds true, making it appear that, mysteriously, a (single) new genotype **G′** has appeared. Panel II shows that, because of canalization for the wild-type in the normal environment **E**, many genotypes ($\mathbf{G_1}$, $\mathbf{G_2}$, etc.) can be consistent with the same phenotype **P**. A transfer to **E′** results in a breakdown of canalization; the genotype-phenotype relationship becomes many-to-many. Selection for **P′** implicitly involves selection for a new *set* of genotypes (**G′**). The large arrows symbolize the shift from one environment to the other and back.

indeed given rise to the appearance of at least one new regulatory gene. A recent replication of the experiment (Gibson and Hogness, 1996) showed that four new regulatory gene combinations had appeared in the course of the assimilation of the four-winged phenotype.

A Model for Genetic Assimilation

Waddington's proposal does not seem to have been tested in an explicitly genetic model. The importance of doing so is obvious: if genetic assimilation can result in a rapid, large-scale change in the phenotype, it would constitute a plausible explanation for major evolutionary changes that have taken place relatively rapidly. My colleague Narayan Behera and I (N. Behera and V. Nanjundiah, unpublished) have developed a model for genetic assimilation. The model depends on a computational algorithm we developed for studying the interaction between phenotypic plasticity and regulatory genes (Behera and Nanjundiah, 1997). Genotypes are represented by two randomly generated strings of genes: a *structural string,* which directly influences the phenotype, and a *regulatory string,* which influences the phenotype indirectly by acting on the functioning of the structural genes. The distinction is more symbolic than real. Depending on the context, the same gene can be thought of as structural or regulatory. To use the language of Larsen (chapter 10, this volume), most genes do double duty as both "worker" and "bureaucrat" genes. Conventional thinking, on the other hand, tends to be comfortable with a nomenclature in which the gene encoding the last protein in a biosynthetic pathway is called a "structural gene," whereas a gene encoding any of the proteins that influence the preceding steps (either directly, as substrates or products, or indirectly, as cofactors, activators, or repressors) is called a "regulatory gene." As far as the following discussion goes, I will use "structural" or "regulatory" merely to refer to the more significant aspect of a particular gene in a particular context.

A structural gene can function constitutively, when it is intrinsically "on" or "off," or facultatively, when it has no intrinsically well-defined state but can function *as if* it were "on" or "off," depending on how it is influenced by the set of regulatory genes that act on it. A structural gene locus has three possible allelic states, **1**, **0**, and **X** (figure 14.5), representing the "on," "off," and "either on or off" states of the gene, respectively, with an **X** finally behaving as if it were a **1** or a **0** (as explained below). Once the state of each **X** is unambiguously assigned, the structural gene string has a phenotype associated with it. There is an optimal phenotype associated with each environment and defined by an appropriate string of **1**s and **0**s. The starting environment is denoted by **A**. To begin with, all individuals express the same phenotype, which we may identify with the wild-type phenotype in Waddington's experiments. In every generation, some individuals are transferred, or exposed at a critical stage of their development, to a different environment, denoted by **B**. Operationally, the transfer increases the likelihood that an individual express a (desired) novel phenotype. Let us imagine

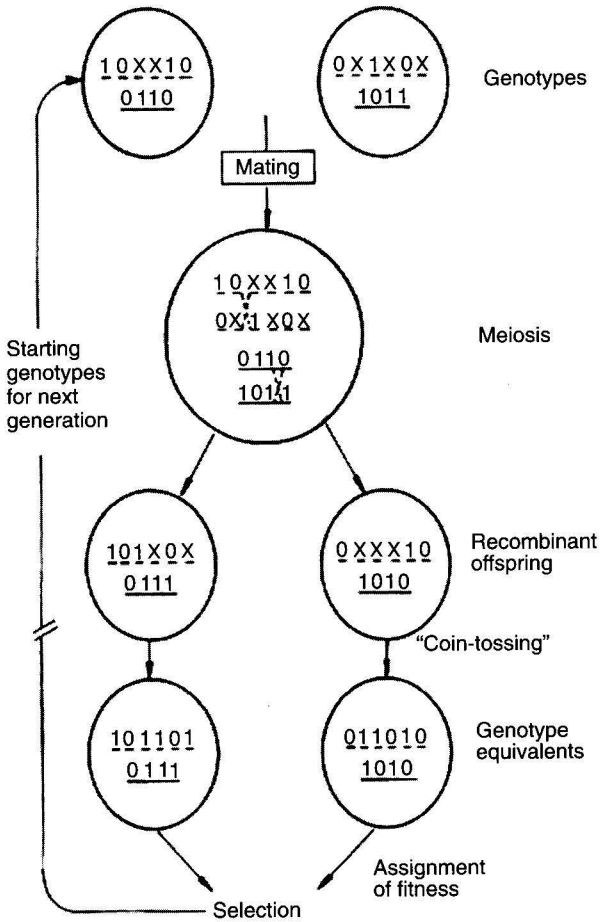

Figure 14.5
Genetic assimilation model, showing two haploid genotypes (small ovals) along with a transient diploid zygote (large oval). Each haploid genotype consists of two strings of genes, a "structural set" (top of each pair, containing **1**, **0**, and **X** alleles) and a "regulatory set" (bottom of each pair, containing only **1** and **0** alleles). Recombinant genotypes are generated via random crossovers occurring during meiosis. By influencing the probabilities of the $\mathbf{X} \rightarrow \mathbf{1}$ and $\mathbf{X} \rightarrow \mathbf{0}$ transitions, the regulatory gene set specifies alternative epigenetic states of the same gene **X**. The transitions result in genotype equivalents, affecting the expression of structural genes and helping to specify the phenotype. "Coin tossing" alludes to the probabilistic basis of the transitions.

that the starting phenotype is that of a normal (wild-type) fly and that the desired phenotype is the full Ultrabithorax phenotype with four wings.

The siblings of those individuals that come closest to expressing the altered phenotype after exposure to **B** are chosen for mating. This is merely in order to demonstrate the versatility of the model; obviously, breeding directly from them would work better. We monitor the success of the procedure in terms of a parameter, **H**, which stands for the fraction of the population that expresses the desired phenotype, either after exposure to environment **B** ($\mathbf{H_B}$) or without any such exposure ($\mathbf{H_A}$). In terms of Waddington's experiment, $\mathbf{H_A}$ is the fraction of the population that expresses the Ultrabithorax phenotype without any prior exposure to ether and $\mathbf{H_B}$ is the fraction that does so after ether exposure. The upshot is that on the whole both $\mathbf{H_A}$ and $\mathbf{H_B}$ increase over the course of generations. In other words, genetic assimilation occurs. As is true of the experimental observation, in environment **B** (i.e., after exposure to ether) a few genotypes give rise to the assimilated phenotype even in the first generation but far fewer—possibly none—do so in environment **A**. The model does not take advantage of all the restrictions that the experiments allow. It makes no assumptions regarding the nature of the two environments, when the shift occurs or how long it lasts. All it says is that the shift from environment **A** to environment **B** permits a broad spectrum of phenotypes to develop. In contrast, the experimental protocols tend to involve a well-defined environmental stimulus applied within a small time window at a specific stage of development. As Waddington found, this approach can cause a rather narrow range of phenotypes to appear. For example, ether treatment favors the "haltere-to-wing" transition, whereas heat shock affects the development of wing veins and leads to the appearance of cross-veinless phenotypes (Waddington, 1961).

Here, in qualitative terms, is how our model works (the explanation is essentially Waddington's). As I have described the system, selection can act only between alternative phenotypes that are correlated with different structural gene combinations. Regulatory genes would seem to play no role in the specification of phenotypes. However, such a conclusion would be false, because the probability that a particular phenotype is actually attained depends on whether an "**X**" is more likely to behave as a "**1**" than as a "**0**," which in turn is influenced by the regulatory genes. Selection acts on the regulatory genes, albeit indirectly, in our model, favoring combinations of regulatory genes that lead to an increase in the probability that an "**X**" will behave as a "**1**." Thus selection leads to the occurrence, through genetic recombination, of regulatory gene sets that ensure that the phenotype corresponding to maximal fitness is attained with a probability close to 1—irrespective of exposure to the source of environmental stress (exposure to ether in Waddington's experiment). In the beginning, environmental stress played a facilitating role by destabilizing the course of normal development and making the appearance of desired phenotypes more likely than would otherwise be the case. At the end, however, the stress is no longer

needed; its role has been made redundant by the canalized action of a new set of genes (figure 14.4).

Evolutionary Possibilities

Phenotypic plasticity can contribute to evolutionary change by two distinct routes, and it is important that we do not conflate them.

In the first route, which I have explored in this article, the original phenotype is canalized in the normal, nonstressful environment, and therefore any underlying genetic variation that exists is cryptic. Stress, whether environmental or genetic in origin, works on the preexisting genetic variation and gives rise to a spectrum of phenotypes, many of them novel, with the optimal phenotype having a higher reproductive success than the others. Sexual reproduction and recombination constantly throw up new arrays of regulatory genes, an obvious prerequisite being that some genetic variation does exist. Selection acts on regulatory gene combinations so as to make the development of the optimal phenotype increasingly probable. To put it differently, selection progressively delinks the appearance of that phenotype from the particular stressful stimulus that potentiated its appearance in the first place. The result is that evolution takes place via genetic assimilation. Epigenetic inheritance is not necessary for this scheme to work; its existence would be an added bonus, as it were. Yet another bonus would be for the optimal phenotype to be produced more or less consistently in the new environment. If that were the case, selection would need to act merely to decrease the variance in the mean level of expression of the optimum.

The second route to evolutionary change, which I have only touched on, could operate in a background of genetic uniformity. Although here, too, phenotypic variation is induced by stress, the cause now lies solely in the manner in which the genotype interacts with the stressful environment. The consequence of the interaction is more than one phenotype. In Bonner's terminology (Bonner, 1965), the consequence is an enlargement in the range variation of the phenotype. Among the phenotypes so generated, the one that leads to the highest reproductive fitness can be rendered constitutive (eventually) via what has been called the "Baldwin effect." In the Baldwin effect, a phenotypic response to a specific environment can become independent of the environment. Baldwin's suggestion was that what started out as a purely physiological adaptation to new conditions could end up, if the right mutational changes took place, as a genetically constitutive outcome (Simpson, 1953). The Baldwin effect may work in situations where the starting population is genetically homogenous and the (stress-induced) variable phenotypes do not differ genetically, at least not in the beginning.

Following a line of reasoning first advanced by West-Eberhard (1986), Stuart Newman (personal communication; also see chapter 13, this volume) has pointed out that it may be useful to broaden the meaning of "genetic assimilation." This could be done by including any set of events whereby a trait which originally depends on an interaction with the environment becomes incorporated into the developmental repertoire of the organism through genetic change. West-Eberhard's (1986) model for major phenotypic change in evolution depends on the ability of more than one phenotype to be consistent with the same genotype or set of genotypes. Her starting assumption is that the same genotype can give rise to distinct but equally well-adapted phenotypes in different individuals belonging to the same population. The alternative phenotypes could persist over generations. Subsequently, perhaps on account of geographical isolation and the demands imposed by a new environment, just one of the alternatives could be favored. The genome would then be "released from the constraints of having to accommodate multiple alternatives," a step that could "facilitate speciation by accentuating divergence from the parent population" (West-Eberhard; 1986, p. 1388).

The less restrictive definition of genetic assimilation may be useful in that it helps us to think in terms of an entire set of phenomena—phenotypic plasticity/epigenetic inheritance/the Baldwin effect/canalization/genetic assimilation—as lying on a conceptual continuum. But by doing so we blur what may be a useful distinction between genetic assimilation and the Baldwin effect; the latter would become just one of the many means through which genetic assimilation could occur. As used here, however, genetic assimilation is quite different from the Baldwin effect. Genetic assimilation requires preexisting genetic variability, whereas the Baldwin effect does not.

In both routes the stressful environment acts as a trigger that permits a whole range of phenotypes to develop. Although any phenotypic trait that thus develops need not be an adaptation to the triggering *condition,* as we have seen, the trait might confer an advantage on its possessor in a quite unrelated environment. In the case of the Waddington-type experiment, the changed environment, **E′** in figure 14.4, is imposed by the experimenter; it is "a peculiar form of predation" (Warburton, 1956, which contains a thought-provoking discussion of this point). Other things being equal, it is reasonable to assume that, for a given intensity of selection, the variance in the mean value of a trait will always be higher when development occurs in a stressful, relatively less tolerant, environment than when it occurs in an environment free of stress. Such an assumption, if true, suggests that selection for a changed phenotype in a stressful environment ipso facto leads to unexpectedly reliable (strongly canalized) development when the original environment is restored (see figure 14.4).

The idea that the extreme phenotypic variation caused by genetic or environmental stress can fuel rapid evolutionary change is not new. Levin (1970) proposed a model for speciation

that involved, as a first step, the isolation of a small number of individuals from a larger population. The smaller group could be located at the periphery of the main population, in a region where the environment was suboptimal and, to that extent, stressful. The smaller group would therefore be subject to strong directional selection. That, combined with phenotypic changes resulting from a failure of reliable development (caused by inbreeding), could lead eventually to the stabilization and fixation of a novel phenotype. In Levin's scheme, environmental stress plays an important role in generating developmental instability, but so does a breakdown of canalization because of inbreeding and the concomitant increase in homozygosity.

Both Levin's and West-Eberhard's proposals postulate phenotypic differences within a common gene pool followed by a subsequent step or steps involving natural selection for a genotype which buffers, or canalizes, the newly favored phenotype. These steps must necessarily involve modifier loci. After canalization has been achieved, the modifiers will mask whatever genetic variation there exists within the population. As a result the underlying genetic variation will become cryptic. (The most famous, not to say famously disputed, discussion of the likelihood of such a course of events is Fisher's model for the evolution of dominance; see Nanjundiah, 1993). But the fact that epigenetic states can be maintained and propagated through many generations of reproduction provides, in principle, yet another means for significant phenotypic variations to arise and evolutionary change to occur, this time without any underlying genetic change—at least none to begin with. The inheritance of epigenetic traits, and, more generally, of acquired traits, excites surprise only when it is observed to occur across individual (organismal) generations. The reason behind this is our ingrained belief in the correctness of Weismann's doctrine of the separation between germ line and soma. But in multicellular organisms, the epigenetic traits expressed by differentiated cells are routinely inherited over many cellular generations. Besides, the germ line–soma distinction breaks down in plants and in many invertebrates.

Finally, we need to address an important question. Why do organisms harbor the capacity to exhibit a large degree of phenotypic variation? One possible answer is that selection for complexity—for genetic networks with high levels of connectivity, feedbacks, and nonlinearities—might automatically render the system susceptible to genetic or environmental stresses. In other words, the capacity to exhibit phenotypic variation may be an inescapable consequence of a complex genome and a complex physiological pathways. Alternatively, as we have also seen, the course of developmental canalization may get disrupted by stress. But there is a third possibility. It may be that for many organisms, the native environments are so variable—"on all scales in space and time" according to Bell (1992)—that natural selection has molded their capacity to exhibit a diverse range of phenotypes when called upon to do so.

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