Colloquium

# Developmental plasticity and the origin of species differences

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**Speciation is the origin of reproductive isolation and divergence between populations, according to the "biological species concept" of Mayr. Studies of reproductive isolation have dominated research on speciation, leaving the origin of species differences relatively poorly understood. Here, I argue that the origin of species differences, and of novel phenotypes in general, involves the reorganization of ancestral phenotypes (developmental recombination) followed by the genetic accommodation of change. Because selection acts on phenotypes, not directly on genotypes or genes, novel traits can originate by environmental induction as well as mutation, then undergo selection and genetic accommodation fueled by standing genetic variation or by subsequent mutation and genetic recombination. Insofar as phenotypic novelties arise from adaptive developmental plasticity, they are not "random" variants, because their initial form reflects adaptive responses with an evolutionary history, even though they are initiated by mutations or novel environmental factors that are random with respect to (future) adaptation. Change in trait frequency involves genetic accommodation of the threshold or liability for expression of a novel trait, a process that follows rather than directs phenotypic change. Contrary to common belief, environmentally initiated novelties may have greater evolutionary potential than mutationally induced ones. Thus, genes are probably more often followers than leaders in evolutionary change. Species differences can originate before reproductive isolation and contribute to the process of speciation itself. Therefore, the genetics of speciation can profit from studies of changes in gene expression as well as changes in gene frequency and genetic isolation.**

speciation | genetic accommodation | adaptive evolution | novelty | parallel evolution

The evolution of reproductive isolation is a defining characteristic of speciation. Reproductive isolation contributes to the diversification of species by creating genetically independent lineages, the branches of a phylogenetic tree. Each branching point of the tree of life is a speciation event. However, reproductive isolation alone does not create a new branch, because by itself it cannot produce the phenotypic divergence represented by the angular departure of a branch from the ancestral form. In the book celebrated by this colloquium, *Systematics and the Origin of Species* (1), Ernst Mayr called phenotypic divergence between populations "the other aspect of speciation." Mayr wrote that speciation has two parts: "One part . . . is the establishment of discontinuities," or reproductive isolation. "The other aspect is the establishment of diversity and divergence, that is the origin of new characters. . ." (ref. 1, p. 23). The origin of species differences, not reproductive isolation, were the main focus of Darwin's book *On the Origin of Species by Means of Natural Selection* (2).

This second aspect of speciation, the origin of new characters, is the subject I address here. In particular, I will pursue Mayr's suggestion that "the workings of this process," the origin of new characters or novel phenotypic traits, "can best be studied if we analyze variation" (ref. 1, p. 23). I will take a close look at the origins of variation, starting with two simple questions. (*i*) Where does the variation, or the variant that makes a new trait, come from? (*ii*) What gets this second, divergence part of speciation, the origin of species differences, started?

## The Nature of Selection and Selectable Variation

The evolutionary synthesis of the mid-20th century, sometimes called the "Neo-Darwinian Synthesis," has been characterized as a synthesis of Darwinism and genetics, with genetic mutation seen as the source of new selectable variation. "The 'genetical theory of natural selection,' the theory that evolution proceeds by natural selection of 'random' mutations, . . . is the basis of the 'neo-Darwinian synthesis'" (ref. 3, p. 187). Consistent with this theory, natural selection, or fitness differences (differential reproductive success), is sometimes defined in terms of genotypes rather than phenotypes (e.g., ref. 4; see also review in ref. 5, Chapter 1). However, the synthesis was not a monolithic affair. Mayr always insisted that the individual phenotype, not the genotype or the gene, is the object of selection (1, 6, 7).

Although the genetic emphasis has been widely adopted, it is an approach that creates problems for understanding the origins of novel traits. The root of the problems is a concept of selection that, mistakenly, requires genetic variation. If selection requires genetic variation, then novel selectable variation must be genetic in nature; hence, mutation is seen as the primary source of evolutionary novelties. However, Darwinian evolution, the origin and evolution of phenotypic traits by natural selection, cannot possibly proceed by natural selection acting directly on mutations or genes. Except for the alleles that carry out their competitive battles within the germ cells for access to the germ line, in processes like meiotic drive, natural selection does not concern reproduction by genes themselves. Most genes under selection depend for their differential propagation on the differential reproduction of the bodies that contain them. That is, genes can replicate themselves, but only within organisms. To spread within populations, they depend on their ability to affect the reproduction of their bearers; they depend on their effects on phenotypes. Therefore, selection should be seen as acting on phenotypes (6), and selectable variation means phenotypic variation, whether it has a genetic component or not. It is adaptive evolution, or a genetic *response* to selection, that requires genetic variation among the selected entities, not selection (differential reproductive success) itself. The question of

a genetic response to selection on environmentally induced traits is discussed below.

Are these trivial, or merely semantic, matters of definition? Different definitions of selection imply different conclusions regarding fundamental issues, such as identification of the units of selection, the importance of development, and how adaptive evolution works. If it is the developmentally organized and environmentally sensitive phenotype that is the object of selection, as argued here, then certain facts that are "surprising" under the conventional mutation-selection idea of adaptive evolution (ref. 4, p. 119) are easily understood and expected (see ref. 5; also see below). Examples include the occurrence of extensive morphological evolution with only a modest number of genetic changes and small genetic changes that have a large effect on the phenotype or fitness (7). If it is the phenotype, not the genotype, that is the object of selection, then selection can proceed for generations without genetic variation and without an evolutionary effect, as long as there is developmentally significant environmental variation. Then, should genetic variation affecting these traits arise, e.g., due to mutation or genetic recombination, it would immediately have an evolutionary effect. Selection on the phenotype means that directional selection can persist over longer time scales than predicted by concepts that see selection as requiring genetic variation. Traits that fail to respond to selection on a short time scale, due to paucity or depletion of genetic variation (e.g., under artificial selection), may undergo evolutionary change over long time scales in nature.

Most important for studies of species diversification, the phenotypic definition of selection permits a more complete analysis of the origins of new traits. If selectable variation is seen to be phenotypic variation, then the scope for the origins of novelty has to be broadened to include environmentally induced phenotypic variation. Phenotype development, which responds to both genomic and environmental inputs, is the source of selectable variation. This analysis brings development, largely omitted from evolutionary biology during the synthesis era (8), to the forefront of evolutionary biology as the source of the variation that fuels natural selection and adaptive evolution.

In this discussion, I look beyond mutation to seek the origins of selectable variation in the developmental plasticity of organisms (for a more extensive discussion, see ref. 5). I argue that the origin of species differences can be explained, and the synthesis of Darwinism with genetics can be improved, by invoking two concepts: developmental recombination and genetic accommodation. Developmental recombination, or developmental reorganization of the ancestral phenotype (5), explains where new variants come from: they come from the preexisting phenotype, which is developmentally plastic and therefore subject to reorganization to produce novel variants when stimulated to do so by new inputs from the genome or the environment. Genetic accommodation, or genetic change in the regulation or form of a novel trait (5), is the process by which new developmental variants become established within populations and species because of genetic evolution by selection on phenotypic variation when it has a genetic component.

Here, I use a broad concept of selection that encompasses both natural and sexual or social selection. I will not extensively discuss these different contexts of selection that are important in driving speciation-related divergence (e.g., see refs. 5, 9, and 10). Instead, I examine the very beginnings of traits and ask how they get started in populations or species.

### The Origin of Divergence: Sequence of Events

A large body of evidence (5) indicates that regardless of selective context the origin of species differences under natural selection occurs as follows:

1. The origin of a new direction of adaptive evolution starts with a population of variably responsive, developmentally plastic organisms. That is, before the advent of a novel trait, there is a population of individuals that are already variable, and differentially responsive, or capable of producing phenotypic variants under the influence of new inputs from the genome and the environment. Variability in responsiveness is due partly to genetic variation and partly to variations in the developmental plasticity of phenotype structure, physiology, and behavior that arise during development and may be influenced by environmental factors, including maternal effects that reflect genetic and environmental variation present in previous generations. Genetic variation and developmental plasticity are fundamental properties of all living things: all individual organisms, with the exception of mutation-free clones, have distinctive genomes, and all of them have phenotypes that respond to genomic and environmental inputs. By "responsiveness" and "developmental plasticity," I do not mean just phenotypic plasticity in the way that term is usually used, to mean only responsiveness to the external environment. Rather, I include responsiveness to the action of genes, which may modify the internal environment of other genes and phenotypic elements within cells, with effects that extend outward to higher levels of organization and responsiveness. Any new input, whether it comes from the genome, like a mutation, or from the external environment, like a temperature change, a pathogen, or a parental opinion, has a developmental effect only if the preexisting phenotype is responsive to it. Without developmental plasticity, the bare genes and the impositions of the environment would have no effect and no importance for evolution.

2. Developmental recombination occurs in a population of individuals because of a new, or newly recurrent, input. A new input from the genome, such as a positively selected mutation, or from the environment of the affected individuals, causes a reorganization of the phenotype, or "developmental recombination." Given the variable developmental plasticity of different individuals, this process produces a population of novel variable phenotypes, providing material for selection.

3. Genetic accommodation may follow. If the resultant phenotypic variation has a fitness effect, that is, it correlates with the survival or reproductive success of the affected individuals, then selection (differential reproduction of individuals or other reproducing entities with different phenotypes) occurs. If the phenotypic variation has a genetic component, selection leads to "genetic accommodation," that is, adaptive evolution that involves gene-frequency change. Genetic accommodation of regulation adjusts the frequency, timing, and circumstances of the novel response (e.g., by adjusting the threshold for its expression), and genetic accommodation of form refines the characteristics and efficiency of the newly expressed trait.

This view of adaptive evolution is conventional in depicting adaptive evolution as phenotypic change that involves gene frequency change under selection. It departs only slightly, but importantly, from the mutation-selection version of adaptive evolution: although novelties may be induced by mutation they need not be; novelties may be induced by environmental factors. In either case, the genetic accommodation of novelty need not await mutation as long as there is a standing pool of genetic variation. As I discuss below, such variation is likely to be sufficient to support a response to selection on virtually any novel trait.

## Developmental Recombination

In developmental recombination, phenotypic traits are expressed in new or distinctive combinations during ontogeny, or undergo correlated quantitative change in dimensions. In the most easily visualized examples, elements of the phenotype controlled by switches are turned off or on in novel combinations. In tropical vines of the genus *Monstera*, for example, a single individual, e.g., of *Monstera dubia*, produces several sequential leaf forms during its ontogeny, and the leaf forms observed in the genus occur in different sequences and combinations in different species, with some species producing several and others only one (5, 11). That is, the leaf forms have been developmentally duplicated, deleted, and recombined in a multitude of ways during the evolution of the genus *Monstera*, giving rise to a variety of species-specific ontogenies. This example illustrates how switch mechanisms can participate in the origins of novelty by recombination of ancestral phenotypic traits. Developmental switches, including decision points in behavior, physiology, and morphology, contribute to the modularity and dissociability of the phenotypic subunits we call "traits" (5), and this modularity, to the degree that it is not constrained by pleiotropic effects of component elements, may allow traits to be expressed in different combinations during development and evolution.

A common kind of developmental recombination is cross-sexual transfer, or the transfer of trait expression from one sex to the other (5, 12–14). The hypothesis that cross-sexual transfer has produced the origin of a novel phenotype is particularly subject to tests because the hormonal mechanisms responsible often can be experimentally manipulated within the same or closely related species. Hormonal correlates of cross-sexual transfer have been studied in some species of birds and mammals where both males and females express parental care (reviews in refs. 5 and 12). In birds, there appears to be a testosterone-mediated tradeoff between male investment in aggressiveness and parental behavior: male parental care is associated with relatively low testosterone levels (15), and in role-reversed species such as sandpipers, where males incubate the eggs, incubation is accompanied by a sharp increase in prolactin (16, 17), as occurs in incubating females (18). In California mice (*Peromyscus californicus*), in which males show all of the parental behavior shown by mothers except lactation, male prolactin levels are similar to those of females soon after parturition (19). Similarly, as in birds, male dwarf hamsters (*Phodopus* sp.) of species showing parental care have elevated prolactin and reduced testosterone during the lactation period, whereas congeneric species in which males do not express male parental care do not show such hormonal changes (20). A key group for research on evolution by cross-sexual transfer in mammals is the voles (*Microtus* species), where there is both intraspecific and interspecific variation in the expression of parental care by the two sexes in a variety of ecological and social circumstances (reviewed in ref. 5).

Cross-sexual transfer also occurs in plants. It has long been recognized as the basis for the origin of the maize ear, which involved the feminization of the male flower, or tassel, of wild teosinte, the ancestor of maize (reviewed in ref. 5). The lateral branches of teosinte were shortened, and the terminal male inflorescence became (or was replaced by) the female ear of maize. This example is especially instructive: the major differences between teosinte and maize are largely explained by the influence of only four or five genetic loci (reviewed in ref. 5, p. 267; see also ref. 21). Leading researchers on the evolution of maize hypothesize that the transition began with environmental induction of branch shortening, followed by genetic assimilation of the short-branched morphology (14, 21). A "catastrophic sexual transmutation" hypothesis (14) drew attention to the contribution of developmental sources of the origin of the distinctive maize phenotype. However, it is impossible to tell, from current information, whether the loci now identified as involved in the change are the result of genetic accommodation based on alleles at low frequency in natural populations of teosinte or are products of later mutations, whose phenotypic effects may have been modified (amplified or reduced) by genetic accommodation. Not all novel elements of the maize phenotype originated simultaneously; there is evidence that increased softness of the glumes originated in Panama (22) rather than in the Balsas valley of Mexico that was the cradle of maize evolution (reviewed in ref. 5).

A different kind of developmental recombination is represented by the famous two-legged goat described in 1942 by the Dutch morphologist Slijper (23), in which a correlated shift in morphology and behavior accommodated an induced abnormality, leading to the well coordinated production of a complex and individually advantageous adjustment, producing a novel phenotype with little or no genetic change (24). Slijper's two-legged goat was born with a congenital defect of the front legs so that it could not walk on all fours, and so it learned to walk and run by using its hind legs alone. Then, when it died an accidental death, Slijper dissected it and documented remarkable changes in muscle and bone, including striking changes in the bones of the hind legs; the leg muscles, including a greatly thickened and elongated gluteal tongue and an innovative arrangement of small tendons, a modified shape of the thoracic skeleton, and extensive modifications of the pelvis (ref. 5, p. 53).

It is not known whether the bipedal goat's abnormal front legs were due to a genetic or an environmentally induced defect, but in either case the inducer acted as a novel switch mechanism that in effect controlled the expression of a whole suite of correlated and adaptive changes in behavior, muscle, and bone. Even though the event that caused these changes was random with respect to adaptation, the phenotypic result was not a random variant. Rather, it was an adaptive accommodation of a random input, the result of pushing to extremes developmental plasticity in behavior, muscle, and bone that had already been subjected to a long history of selection and adaptive evolution. Similar effects on behavior and morphology are quite common in quadripedal mammals, including primates, forced or trained to walk upright (ref. 5, p. 42, figure 3.12 on bipedal baboon; ref. 25; see also descriptions of a bipedal macaque in ref. 62 and a bipedal dog in ref. 63). These observations raise the possibility that the two-legged-goat effect, or "phenotypic accommodation" (5, 26), has played a role in the evolution of bipedal locomotion in vertebrates, including humans, as suggested by Slijper, who noted that some of the novel morphological features of the two-legged goat resembled those of kangaroos and of other bipedal species such as orangutans (23). Japanese macaques experimentally taught to walk upright develop human-like gait characteristics (25), suggesting that the evolution of bipedalism in humans might not be as difficult or as large an evolutionary step as some anthropologists have believed. The distinctive anatomical features of humans compared with other primates that are associated with bipedal running include changes in muscle mass, tendon length, and thorax and pelvis shape (27), the same features that underwent striking alterations in the bipedal goat (23). It is highly likely that developmental plasticity contributed to the species-specific morphological changes associated with the evolution of human bipedal walking and running.

Developmental recombination that can result in evolutionary divergence can occur at all levels of organization. At the molecular level, the modular structure of protein molecules, which parallels the modular organization of switch-controlled phenotypic development, facilitates reorganization. Proteins are composed of domains associated with the exon (expressed)

regions of DNA that produce them. Some domains are known to be associated with particular biochemical functions, in a manner that parallels the functional and structural modularity of other aspects of the phenotype (5). The fibronectin family of proteins is a good example of a set of related proteins, where the nine domains or subunits that compose them are duplicated and organized in different combinations to form different molecules with distinctive functions (28).

## Developmental Recombination and Parallel Species Pairs

Recurrent phenotypes, similar or identical phenotypic traits with discontinuous phylogenetic distributions, are quite common in a wide diversity of taxa (5). Their similarity is sometimes attributed to parallel evolution, the independent origin of phenotypic similarity due to selection and adaptive change in similar environmental conditions. Interpretation of the origin of species differences in stickleback offers a good example. In three-spined stickleback (*Gasterosteus aculeatus*), a complex of closely related species, there is a recurrent set of species pairs, with one member of the pair a slender, large-eyed "limnetic" form, which typically lives in the water column and feeds on plankton, and the other a stockier, smaller-eyed "benthic" form, typically a bottom dweller. All of these parallel species pairs are derived from anadromous ancestors, which migrated between the sea and the freshwater of rivers and lakes.

According to the way biologists usually think about the evolution of similarity, the recurrence of species pairs like these are the result of parallel evolution by natural selection on reproductively isolated breeding populations in parallel environments (29). By that interpretation, the anadromous ancestor gave rise repeatedly to the parallel forms by recurrent independent adaptation to parallel ecological conditions within lakes. The benthic and limnetic body types are invented over and over again independently, due to natural selection in a pair of ecological conditions that are common in lakes.

Developmental recombination offers an alternative explanation for parallelism in stickleback (5, 30). The recurrence of this pair of forms suggests that there may be something about the development of their common ancestor that enables it to give rise to these two particular forms readily, by means of altered expression of ancestral traits. Research on the ontogeny of anadromous stickleback (31) has revealed that individuals are limnetic when young. They have a slender body form and live in the water column, where they feed on plankton like a limnetic species. Older individuals look and behave like the benthic form, with a stockier body and bottom-feeding habits. So the ancestral population occupies both of the habitats observed in the descendent species pairs and exhibits both phenotypes at different times during its life cycle, a pattern that suggests that the different recurrent forms may have originated not by parallel evolution but by altered timing (heterochrony) in the expression of previously evolved adaptive traits (5). By the heterochrony hypothesis, the limnetic-form species have juvenilized adults, and the benthic-form have full-sized adults like those of the ancestral form.

The morphology and behavior of stickleback are highly plastic, with feeding behavior influenced by learning (32). Feedback between morphology, food, and habitat choice, and learned feeding specializations would speed divergence between limnetic and benthic forms. When individuals of a sympatric benthic and limnetic pair of species were reared on each others' diets, their morphologies changed toward increased resemblance to the species whose diet they had experienced (33).

This discussion is not to deny the importance of natural selection in contrasting habitats for the evolution of species differences and their possible effects on the origin of reproductive isolation (ref. 10; also see below). Rather, it is to urge a deeper look at the question of how such differences originate in

lineages with a developmentally and adaptively versatile ancestral phenotype. Obviously, selection in their respective habitats would play an important role in the fixation and elaboration of the divergent descendent stickleback forms, but their parallel morphologies and behaviors may have originated before speciation. The plasticity hypothesis is supported by the fact that in all of the fish genera with replicate speciation between limnetic and benthic species pairs, the limnetic–benthic interspecific alternatives occur as intraspecific alternative phenotypes as well, in at least some populations, suggesting that they, too, could have originated as intraspecific developmental variants. These genera include, in addition to stickleback, lampreys (*Lampetra*), arctic charr (*Salvelinus*), Pacific salmon (*Oncorhynchus*), lake whitefish (*Coregonus*), trout (*Salmo*), and smelt (*Osmerus*) (reviewed in ref. 5).

Replicate speciation has produced three parallel species pairs of 13- and 17-year periodical cicadas (*Magicicada* species), with one of the 17-year populations (*M. septendecim*) giving rise to a 13-year species (*M. neotredecim*), forming a species pair (*septendecim–neotredecim*) within a species pair (*tredecim–septendecim*) (34). The recurrent phylogenetically abrupt switches between these two life cycles suggests that, as in the replicate species pairs of fishes, some ancestral developmental mechanism with a 4-year periodicity has repeatedly influenced life-cycle divergence between the species pairs of periodical cicadas (5, 35), although the physiological mechanism is poorly understood.

There are a great many examples of phylogenetic recurrence in a wide diversity of animals and plants (5). Phylogenetically separate, recurrent phenotypes show that a common type of developmental recombination is the reexpression of phenotypes that have been lost because of developmental deletion, alteration of a regulatory mechanism without extensive alteration of other aspects of the developmental capacity to produce the lost form. As a rule of thumb, recurrent parallel forms suggest ancestral developmental plasticity for producing both forms. This phenomenon may explain many of the parallelisms and homoplasies that are so commonly discovered in systematics and phylogenetics, but it is a hypothesis that needs to be tested case by case using detailed comparative studies of phenotypic variation and its developmental basis.

## Gene-Expression Consequences of Developmental Recombination

Individual development can be visualized as a series of branching pathways. Each branch point is a developmental decision, or switch point, governed by some regulatory apparatus, and each switch point defines a modular trait. Developmental recombination implies the origin or deletion of a branch and a new or lost modular trait. It is important to realize that the novel regulatory response and the novel trait originate simultaneously. Their origins are, in fact, inseparable events: you cannot have a change in the phenotype, a novel phenotypic state, without an altered developmental pathway.

In terms of gene expression, developmental recombination means that a set of ancestral genes are now coexpressed, or their products used, in a new combination or a new context, and the ancestral regulatory mechanisms are now triggered by a new inducer or an old one in some new sequence or environmental context.

Although the subject of developmental regulation may bring to mind "regulatory genes" or "master control genes," such as hox genes, regulatory mechanisms controlling the expression of many of the traits I have discussed here must often be polygenic in nature, because they may involve complex aspects of the phenotype, including neural and sensory equipment, hormone systems, and complexly responsive tissues and organs. Polygenic complexity of regulation increases the likelihood that there will

be genetic variation in the ability to respond and, thus, that genetic accommodation will occur. Similarly, there is likely to be polygenic variation in the dimensions and subcomponents of a trait that are newly expressed together because of the developmental recombination of ancestral traits. It is these two sets of coexpressed genes that are exposed as sets to selection: those that modify the regulation of expression of a trait and those that are activated as a set, or whose products are used together as a set, to affect the form of a trait.

## Genetic Accommodation

Genetic accommodation is simply quantitative genetic change in the frequency of genes that affect the regulation or form of a new trait. If a novelty has been induced by a mutation, then the mutation would be, at least initially, a gene of major effect on a polygenic regulatory mechanism. If regulation is polygenic, as just described, then the effect of that mutant gene would be subject to genetic modification involving multiple loci.

The sensitivity of a regulatory mechanism can be adjusted either up or down. If a novel trait is favored by selection, then genetic accommodation is expected to lower the threshold for its production or increase liability to pass the threshold, and genetic assimilation or fixation of the trait may occur. If the trait is selected against, then genetic accommodation would raise the threshold or reduce the liability for its expression until it is not expressed at all. Still another possibility is that a novel trait may persist indefinitely as an adaptive alternative phenotype. The important point here is that the genetic accommodation of regulation can determine the frequency of the trait in a population. Frequency of expression does not depend on the frequency of the inducer (mutation or environmental factor) alone.

For these reasons I consider genes followers, not leaders, in adaptive evolution. A very large body of evidence (5) shows that phenotypic novelty is largely reorganizational rather than a product of innovative genes. Even if reorganization was initiated by a mutation, a gene of major effect on regulation, selection would lead to genetic accommodation, that is, genetic change that follows, and is directed by, the reorganized condition of the phenotype. Some authors have expressed this pattern as "phenotype precedes genotype" (36). This description applies best to genetic assimilation, a special case of genetic accommodation that begins with environmental induction and proceeds toward fixation of the novel trait (37).

Of course, genetic mutation must ultimately fuel the genetic variation that permits adaptive evolution. However, genetic accommodation need not await mutation. There are two kinds of evidence that the standing genetic variation (38) of particular responses is probably usually sufficient to support genetic accommodation without mutation. A large accumulation of data on protein polymorphisms has shown that genetic variation is common in natural populations (39); and virtually every trait subjected to artificial selection shows a response to selection (references in ref. 5). A rare class of exceptions occurs under artificial selection for directional (consistently right or left) asymmetry of various traits (ommatidia number, wing folding, eye size, and thoracic bristle number) in *Drosophila* (40–43). Nonetheless, directional (consistent right or left) asymmetry has evolved repeatedly in insects (e.g., see ref. 44 on genitalia and other abdominal structures) and in other organisms (36), suggesting that even this category of lack of response to selection can be overcome by variation and selection during longer evolutionary time scales. The phenotypic definition of selection helps to explain some of these cases, in which lack of genetic variation initially may have blocked a response to positive selection, and yet the trait eventually evolves to fixation. Because selection (differential reproductive success) of phenotypes does not require genetic variation, directional selection can persist generation after generation, favoring either the right or left form under environmentally influenced fluctuating asymmetry ("antisymmetry"), a

state known to precede the evolution of some examples of directional symmetry (36). Then an evolutionary response to selection would occur as soon as favorable genetic variations arise, e.g., due to mutation. Thus, although standing genetic variation usually must be sufficient to produce a response to selection (38), genetic accommodation may in some cases, like that of directional asymmetry, await mutation (36).

## Evolutionary Potential of Environmentally Induced Change

Biologists are inclined to doubt the evolutionary importance of environmentally induced traits because it is not immediately obvious how they can be inherited in subsequent generations. Initiation by mutation is intuitively more appealing because it solves the problem of novelty and heritability in one stroke. However, environmentally induced variants are heritable as well, insofar as the ability to respond by producing them is heritable (that is, genetically variable). The responsiveness of organisms to environmental influence involves mechanisms that are likely to be genetically complex and therefore subject to genetic variation at multiple loci, as just discussed.

A mutant gene may seem more dependable, that is, more likely to persist across generations, than a novel environmental factor. However, it takes only a little reflection on the nature of development to appreciate the dependability of environmental factors. All organisms depend on the cross-generational presence of large numbers of highly specific environmental inputs: particular foods, vitamins, hosts, symbionts, parental behaviors, and specific regimes of temperature, humidity, oxygen, or light. Such environmental elements are as essential and as dependably present as are particular genes; some, such as photoperiod and atmospheric elements like oxygen and carbon dioxide, are more dependably present than any gene in particular habitats and zones, so we forget that these environmental factors constitute powerful inducers and essential raw materials whose geographically variable states can induce developmental novelties as populations colonize new areas.

Some environmental elements act as developmental building blocks and signals quite comparable to the products of genes. For example, DNA microarray studies have shown that environmentally supplied bacteria in the digestive tract of zebrafish regulate the expression of 212 genes. Without these bacteria, many of which produce highly specific host responses, the developing fish die (45). Given the evidence, familiar to everyone, that numerous environmental inputs are consistently supplied (essential) during normal development, the skepticism of biologists regarding the reliability of environmental factors relative to that of genes has to rank among the oddest blind spots of biological thought.[†]

Contrary to the notion that mutational novelties have superior evolutionary potential, there are strong arguments for the greater evolutionary potential of environmentally induced novelties. An environmental factor can affect numerous individuals at once, whereas a mutation initially can affect only one. The larger the population affected, the greater the likelihood that an environmentally induced novelty occurs in a favorable genetic, phenotypic, or selectively advantageous environment in at least some subpopulation of the individuals affected, and the larger the probability of genetic variation that can result in an evolutionary response to selection. Furthermore, environmentally induced phenotypes can persist over generations even when disadvantageous because, in contrast to mutations, they cannot readily be eliminated by selection. The superior persistence of

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[†]The assumption that genetic inputs are more reliable than environmental ones may be further challenged by future research on gene expression. Recent findings suggest that processes like gene transcription, involving small numbers of molecules, are especially subject to randomness and noise (46).

environmentally induced traits allows time for genetic accommodation and adaptive evolutionary change. For example, undersized and "starvation" forms are exceedingly common in nature, even though disadvantageous, because developing individuals often cannot escape environmental variation in food supply. As a result, evolved specializations to small size, such as nonfighting morphologies and behaviors of small males (5), and other striking size-associated adaptations (47) are common in nature.

Population-wide environmental induction and genetic accommodation sometimes occur when populations colonize islands, where new environmental stimuli and opportunities repeatedly induce novel phenotypes, such as learned foraging techniques, which then subject the population to selection on associated morphology, behaviors, and diet-associated physiology (5, 48) and when habitat change forces dietary change in ingested carotenoids, with effects on the plumage colors and associated evolved biochemistry of birds (48).

### The Origin of Species Differences: Before or After Reproductive Isolation?

Trait origin by developmental recombination predicts several properties of species and their genetics as follows:

(*i*) In evolution by developmental recombination, the same genes are used over and over in different contexts and combinations. This process should contribute to the maintenance of the same or homologous genes over long periods of evolutionary time and would help account for finding similar genes in distantly related species or the observed conservation of genes across long periods of evolutionary change;

(*ii*) Small genetic distances between species with strikingly different phenotypes are expected because extensively reorganized new phenotypes can occur with little genetic change;

(*iii*) Homoplasy and parallelism are expected to be common among related species, because developmental flexibility can give rise repeatedly to the same kinds of variation; and

(*iv*) Phenotypic differences that eventually distinguish species may often arise before the advent of reproductive isolation between them, because the origin and maintenance of more than one developmental pathway can occur within a population; the evolution of a divergent novelty does not require gene-pool divergence, only developmental-pathway and gene-expression divergence (5).

This last point is important for students of systematics and speciation because it means that some phenotypic divergence assumed to mark species may in fact represent intraspecific alternative phenotypes or, in paleontology, morphotypes assumed to be species when they are in fact complex alternative forms that represent gene-expression, not genetic, differences between individuals. More importantly for the process of speciation, divergent developmental pathways within species enable the exploitation of different conditions and resources by members of the same species as adaptive options, and assortative mating by developmentally similar individuals can then contribute to speciation, whether in sympatry or in geographically isolated populations. In either setting, selection for a single alternative would speed the specialization of an increasingly monomorphic subpopulation, because an approach to phenotypic fixation is expected to accelerate the (genetic) evolution of the fixed form (5, 49). This acceleration would contribute to the evolution of reproductive isolation between populations with different alternative phenotypes, insofar as genetic divergence contributes to the likelihood of pre- or postzygotic reproductive incompatibility between them (for tests of this largely unexamined condition, see ref. 50).

Consistent with the hypothesis that ancestral developmental alternatives can precede and contribute to speciation, there are many species differences that parallel differences between alternative phenotypes within closely related species (5, 51, 52). Multiple kinds of evidence support the hypothesis that species differences originated before reproductive isolation in a variety of organisms, including buttercups, butterflies, aphids, migrant fishes and birds, and socially parasitic ants (reviewed in ref. 5). Intraspecific divergence in host-specific behaviors and life-history characteristics has repeatedly been suggested as possibly contributing to speciation in apple maggot flies (*Rhagoletis*) (5, 53, 54), but the possible contribution of preisolation developmental divergence in the form of a host-associated polyphenism or polymorphism has never been systematically investigated in that genus. Despite the experimental evidence for speciation-related preisolation phenotypic divergence in host-switching phytophagous insects, given long ago by Walsh (53) and mentioned by Bush (55), speciation research has focused primarily on genetic divergence accompanying or following breeding isolation in *Rhagoletis* and other organisms.

Research like that of Schluter and associates (10, 29) shows how traits such as body size, which have diverged under natural selection, can contribute to the origin of reproductive isolation. Variation in body size is often associated with the evolution of condion-sensitive, faculatatively expressed alternative phenotypes within species, including in fishes and other organisms, such as ants, where size differences have been implicated in the origins of reproductive isolation between contrasting phenotypes (summary in ref. 5). Such phenotypes, like those of geographic isolates, evolve under natural and sexual or social selection, but with their divergence originally mediated by developmental plasticity (5). Indeed, speciation-related divergence is arguably facilitated, that is, more rapid and readily divergent, within species than between geographic isolates (5, 9, 51). Therefore, hypotheses such as the "ecological speciation" hypothesis (10), which seek to associate preisolation divergence under selection with the origin of reproductive isolation, whether in sympatry or allopatry, may find some of their best support in taxa containing marked intraspecific alternative phenotypes ("ecophenotypes," polyphenisms, polyethisms, etc.).

At present, it is impossible to evaluate the contribution of preisolation divergence by means of developmental plasticity to the evolution of reproductive isolation because genetic studies have been designed to detect the extent and breeding consequences of differences between populations, by means of studies of genetic distances, hybridization, etc. (e.g., see review in ref. 42) rather than their possible sources in patterns of gene expression within populations. Preisolation divergence may turn out to be another "excellent but embarrassing example of not being able to find what you are not looking for" (56), because there is plenty of indirect evidence that developmental plasticity has been important for the origins of species differences. Not only are species differences often parallel to intraspecific alternative phenotypes, as already mentioned, but the rampant speciation and associated phenotypic diversification that characterize some of the most spectacular adaptive radiations known can be linked to particular kinds of developmental plasticity. The beak and trophic diversification of Darwin's finches in the Galapagos islands has involved learned associations between beak size and shape and feeding preferences; the niche diversification of African lake cichlids is associated with dietary flexibility in mouth morphology and behavior; and the larval habitat diversification of Hawaiian *Drosophila* may have involved biochemical versatility within species (reviewed in ref. 5).

### Future of Genetic Studies of Speciation

Inspired by Dobzhansky and the Darwinian evolutionary geneticists Fisher, Haldane, and Wright, as well as by Mayr's biological

species concept (6) and other concepts that also emphasize the role of genetic isolation in promoting genetic divergence (57, 58), research on species differences inspired by the synthesis has focused on the genetics of reproductive isolation between populations. This approach has produced many insights regarding the process of speciation, but it has created a kind of selective vision that may sometimes overlook the potential contribution of preisolation phenotypic divergence by means of developmental plasticity and its possible consequences for assortative mating and reproductive isolation. Ironically, this emphasis on genetic isolation may impede understanding of the causes of speciation because important gene-pool or genotypic-cluster (57) differences may come to exist only when reproductive isolation is already well advanced. So geneticists may end up describing the results of speciation rather than its causes.

Lack of attention to developmental phenomena in relation to speciation promises to change, because genomic studies of speciation can now contemplate gene-expression as well as gene-frequency data (e.g., ref. 30 on the stickleback model system and ref. 59 on *Drosophila*). Research on patterns of gene expression makes it possible to pinpoint the (expressed) loci that are actually subject to selection in the evolution of species differences, beginning with differences that arise because of developmental recombination without reproductive isolation. Comparative genomics has the potential to illuminate the contribution of developmental-genetic processes to speciation. Are the alternative sets of genes that are differentially expressed in particular forms in an intraspecific polyphenism, polymorphism, or life-stage difference, like those observed in some host-specific insects or during the ontogeny of stickleback, the same sets of genes that characterize differences between recently derived host races or species whose phenotypes parallel those forms? Is there a burst of genetic change in the modifiers of form when a particular form in a polyphenic population approaches fixation, as predicted by theoretical models (5)? Finally, does the accompanying phenotypic change contribute to the evolution of reproductive isolation?

As genomic libraries expand and the associated techniques for research on gene expression become increasingly accessible to speciation biologists (e.g., see refs. 30 and 58), the interests of geneticists will increasingly converge with those of organismic biologists interested mainly in phenotypes (60). The meeting ground of intermediate processes, such as hormone physiology, neurobiology, and subcellular signaling process (61), topics long estranged from direct involvement in speciation studies and other areas of evolutionary biology, then will be of increasing interest, because these are the processes that link environmental, genetic, and phenotypic variation to selection and evolution through their mediation of gene expression. Progress in understanding the developmental nature of variation supplies a missing piece of the synthesis begun by Dobzhansky and Mayr.

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