

Developmental Constraints, Modules, and Evolvability

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ABSTRACT

This chapter discusses the developmental origins and evolutionary implications of covariation between traits. These are important factors influencing the evolutionary potential of morphological traits. Strong covariation can constitute an evolutionary constraint because some character combinations are more likely to evolve than others. Modularity is a widespread feature of organismal organization: Groups of traits covary with each other but are relatively independent of other groups of traits. This modularity results from a similar organization of developmental

systems, where signaling interactions primarily take place within spatially distinct fields. The covariation among morphological traits can result from direct interactions of the developmental pathways that produce the traits, which take place within developmental modules, or from parallel variation of separate pathways in response to the simultaneous influence of an external factor. These two origins of covariation among traits have different implications for pleiotropy of genes, the evolution of pleiotropy, the total genetic covariance structure, and the resulting evolutionary constraints.

INTRODUCTION

The question about the relative importance of intrinsic and extrinsic factors as determinants of evolution has existed throughout the history of evolutionary thought and has led to lively debates under different headings (Gould, 2002). In recent years, this debate has mainly concentrated on the question of whether natural selection primarily drives evolution, which would therefore proceed by adaptive optimization of characters, or whether the direction and rate of evolution are affected substantially by factors intrinsic to the organism and its function (Gould and Lewontin, 1979; Alberch, 1989; Parker and Maynard Smith, 1990; Rose and Lauder, 1996; Gould, 2002). With the increased interest in the influence of developmental processes on evolutionary change (Kirschner and Gerhart, 1998; Arthur, 2001), a new perspective on this topic has opened up, and questions have been asked about evolvability, the intrinsic tendency of organisms to produce new variation (Wagner and Altenberg, 1996). Are all features of the phenotype equally variable, or are there features that are inherently more variable than others and therefore more likely to evolve rapidly? If there is such a tendency of organisms to produce particular kinds of variation, does it evolve itself?

A topic that has long attracted particular attention is the integration among the traits of an organism (Olson and Miller, 1958; Cheverud, 1982, 1996; Wagner, 1996). The central question is whether variation is coordinated among traits so that each organism is a fully integrated ensemble or whether the organism is a composite of several subunits that are more or less free to vary independently from one another. Strong integration of parts may lead to an organism that is functionally better coordinated. However, such global coordination may limit the potential for future evolution because different functional systems cannot evolve separately. Each adaptive improvement would come at the cost of a deterioration of some other aspect of performance (Kirschner and Gerhart, 1998).

In this chapter, I examine the topics of evolvability and constraint as well as integration and modularity from a perspective that unites development and evolutionary quantitative genetics. Much of the emphasis is on the question of how

integrated morphological variation originates in the development of the respective traits. The explicit consideration of the developmental origin of morphological covariation (e.g., Riska, 1986; Klingenberg, 2004) offers a possible alternative to the hypotheses on the origin of integration and modularity by adaptive evolution (Cheverud, 1984, 1996; Wagner, 1996; Wagner and Altenberg, 1996). I argue that the organization of morphological structures into developmental modules is a crucial factor for the genetic architecture of these traits and the evolution of genetic covariance structure.

I. EVOLVABILITY AND CONSTRAINTS

Evolvability is the potential of a population to respond to selection or to undergo nonadaptive evolution by drift. By contrast, a constraint is something that limits or biases the potential for evolutionary change, and it is therefore a force opposing or channeling evolution, reducing evolvability in at least some directions of the phenotypic space (e.g., Maynard Smith *et al.*, 1985).

Most of the discussion on constraints has focused on their role in limiting the evolvability of traits in particular directions, making certain phenotypes inaccessible to evolution or at least more difficult to attain. In contrast to this predominant usage, Gould (1989; 2002, pp. 1025–1061) went to considerable length to emphasize that constraint is not necessarily a “negative” force limiting or reducing the evolvability in some directions of phenotypic space, but that it can be a “positive” force biasing or channeling evolutionary variation toward certain directions. As prime examples of constraints, Gould (2002, pp. 1037–1051) mentions allometry and heterochrony, for which ontogeny provides a strong directionality in the phenotypic space, because evolution by changes of growth control can easily achieve an extension or truncation of an ancestral growth trajectory (e.g., Klingenberg 1998). Evolution by this mechanism will therefore be constrained (in the positive sense) to changes in the direction of a conserved ontogenetic trajectory, but it is also constrained (in the negative sense) from achieving changes in directions perpendicular to this growth trajectory. Arthur (2001) suggested the term *developmental drive* for the positive sense of constraint. Note, however, that this drive is bidirectional, because variation is biased to be along a particular axis in space, but that variation along this axis is equally distributed in both directions and not just in one of them, as is the case for other kinds of drive that are unidirectional, for instance, meiotic drive in population genetics (Hartl and Clark, 1997, pp. 247–250) or the different types of drive in macroevolution (Gould, 2002, pp. 717–731).

Constraints or biases of variation can be absolute or relative (Figure 11-1). An absolute constraint is the situation where there is no variation at all in some directions of phenotypic space, and all variation is contained entirely in a subspace,

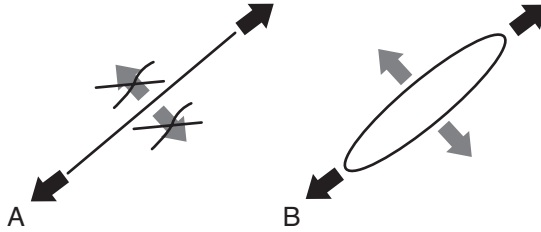


FIGURE 11-1. Constraints on phenotypic variation. (A) Absolute constraint. There is no variation in one direction of the phenotypic space, the plane of the graph. Evolution can therefore only occur along the single axis of variation (black arrows), but not in the direction perpendicular to it. (B) Relative constraint. There is more variation in the direction of the long axis of the ellipse, and the phenotype is therefore more evolvable (black arrows) than in the direction perpendicular to it (gray arrows).

for example, in a single line in a two-dimensional graph (Figure 11-1A) or in a plane of a three-dimensional space. In this case, phenotypes lying outside the subspace are inaccessible to evolution. An absolute constraint will always be associated with a gap in the distribution of traits or with a clear limit of that distribution (Alberch, 1989).

An interesting way to examine whether a “missing” morphology may result from an absolute constraint is to examine developmental anomalies such as teratologic and mutant phenotypes. Because these phenotypes are clearly not functional and often lethal, the regularities in the phenotypes are caused by the developmental system, and selection for functionality can be ruled out. These regularities and patterns can reveal the system’s potential to generate novel forms, which is a precondition for evolvability, and may reveal a “logic of monsters” (Alberch, 1989). Similarly, mutants can be used to “engineer” novel phenotypes and to explore the limits of developmental systems. Dworkin *et al.* (2001; see also Larsen, 2003) combined *Drosophila* mutants to flies with antennae resembling the biramous condition seen in the limbs of crustaceans and ancestral arthropods. The problem with this approach is, of course, whether such an engineered condition really corresponds to a purportedly constrained morphology, that is, whether the boundaries that define absolute constraints have been identified correctly.

Relative constraints (Figure 11-1B) concern differences in the amounts of variation available in different directions of the phenotypic space. The question is not whether or not a particular phenotype can be reached at all, but how easily a population will evolve in different directions of the phenotypic space. If selection for a single optimal phenotype is maintained for a sufficient number of generations, a population will eventually overcome relative constraints to achieve the phenotypic optimum. The importance of relative constraints is therefore not primarily in precluding particular phenotypes as the endpoint of selection, but such constraints are a major factor determining the evolutionary trajectory by which the population

will reach this endpoint. If several alternative optima exist as separate peaks of the fitness landscape, relative constraints may be decisive in determining which peak the population will reach.

Absolute constraints are fairly rare, because most phenotypic traits show considerable genetic variation (e.g., Roff, 1997). However, no systematic searches for absolute constraints have been done with rigorous multivariate methods. Such searches face considerable technical and statistical difficulties so that large experimental designs will be required. Weber (1990, 1992) applied artificial selection for different shape changes in *Drosophila* wings and obtained significant responses for all of them. Likewise, Beldade *et al.* (2002b) conducted selection experiments on the relative sizes of different eyespots on butterfly wings and found a substantial response to selection, even for the trait combinations that initially had been expected to be constrained.

The structure of genetic variances and covariances reflects these constraints (Lande, 1979; Cheverud, 1984; Arnold, 1992; Roff, 1997; Stepan *et al.*, 2002). A variety of experimental designs is available to estimate the genetic covariance matrix (**G** matrix), which contains the genetic variances and covariances among the traits of interest (e.g., Lynch and Walsh, 1998). Constraints can therefore be diagnosed and quantified by analyzing the **G** matrix with the methods of multivariate statistics.

Absolute constraints can be shown if the **G** matrix has one or more directions that are devoid of genetic variation (Figure 11-1A), or in algebraic terms, if the **G** matrix is singular. There are several statistical tools to assess this condition. A method that is available in most standard statistical software packages is principal component analysis (e.g., Jolliffe, 2002) of the **G** matrix: If there are one or more principal components that do not account for any variation (eigenvalues of zero), there will be absolute constraints. The reverse is not necessarily true, however, because absolute constraints may not be linear. The presence of nonlinear absolute constraints may not yield a singular **G** matrix. Such nonlinear absolute constraints may be difficult to demonstrate empirically. In general, given the considerable difficulties involved in estimating **G** matrices (Lynch and Walsh, 1998), inferring genetic constraints from them is a challenging task. It may be helpful to note, therefore, that absolute constraints also can be inferred if the phenotypic covariance matrix (**P** matrix) is singular, because this automatically implies that the **G** matrix must be singular. It usually will be easier to show that there are principal components of the **P** matrix that are not associated with any variation, although this method cannot reveal all cases where the **G** matrix is singular. All these inferences about the **G** matrix are technically demanding in their application.

Relative constraints result from a situation in which the amounts of variation in different directions of the phenotypic space are unequal (Figure 11-1B). Accordingly, the propensity for evolutionary change by selection will be greater for some trait combinations than for others, and evolutionary change by drift will tend

to favor the same trait combinations. Relative constraints can be assessed by the differences of the eigenvalues of the G matrix. There are strong relative constraints if some principal components take up a disproportionate share of the total variation, whereas others account only for minor amounts of variation (e.g., Klingenberg and Leamy, 2001). Because the response to natural selection depends on the P and G matrices jointly as well as the direction in which selection is applied, the relative magnitudes of eigenvalues in either matrix alone are not always sufficient to assess the severity of constraints.

As can be seen from the diagrams in Figure 11-1, the constraints result from an association between the coordinates of the plots. For absolute constraints, one or more variables are completely determined by the remaining ones, and not every combination of traits is available. For instance, if all the variation is on a single straight line in a plane, then any phenotype that is at a distance to the line is inaccessible (Figure 11-1A). In the case of a relative constraint, however, this association is a stochastic one, where the value of one variable can only be predicted from the value of the other variable or variables with some uncertainty (Figure 11-1B). For both absolute and relative constraints, associations of traits are of critical importance, because it is covariation between traits that limits evolution in some directions and makes certain combinations of traits difficult to achieve. Therefore adaptive evolution of individual traits can be constrained by the integration of traits within the entire organism.

II. INTEGRATION AND MODULARITY

The idea that the parts of organisms are coordinated to form a functional whole goes back to the early nineteenth century when Cuvier stated it as the “principle of correlation” (e.g., Mayr, 1982, p. 460 f.) and more recently has been discussed under the heading of morphological integration (Olson and Miller, 1958; Cheverud, 1996; Hallgrímsson *et al.*, 2002). This coordination has a functional and possibly adaptive basis as well as a developmental and mechanistic basis, and the two contexts are often distinguished by terms such as *functional integration* or *developmental integration*. Here, I will discuss primarily the developmental basis of morphological integration, and functional aspects will play only a secondary role.

Organisms are not completely and equally integrated throughout, but they are organized into distinct parts, or modules (Figure 11-2). Modules are assemblages of parts that are tightly integrated internally by relatively many and strong interactions but relatively independent of one another because there are only relatively few or weak interactions between modules (Cheverud, 1996; Raff, 1996; Wagner, 1996; Wagner and Altenberg, 1996). The development and morphology of different modules can therefore evolve independently, at least to some extent, without disrupting function at the level of the whole organism. This modular type of

organization recurs at different levels from the interactions in genetic regulatory networks to the morphological structure of whole bodies and their parts (e.g., von Dassow and Munro, 1999; Winther, 2001). In this chapter, the focus will be this organismal level of organization, where modular structure results from interactions among the developmental processes that build morphological parts (Cheverud, 1996; Klingenberg, 2003a; Klingenberg *et al.*, 2003).

Modularity is a hierarchical concept—there can be modules within modules, depending on the level of organization (e.g., in Figure 11-2, modules 1 and 2 together constitute the higher-level module 4). For instance, this structural hierarchy can reflect successive rounds of patterning that progressively subdivide the developmental field into finer regions corresponding more and more to the anatomic details of the prospective morphological structure (Davidson, 1993; Wilkins, 2002, Chapter 8). As a consequence, modules arising in this manner at a later time are therefore within modules that have originated earlier. The subdivision of embryonic fields is not the only process giving rise to modularity, and processes

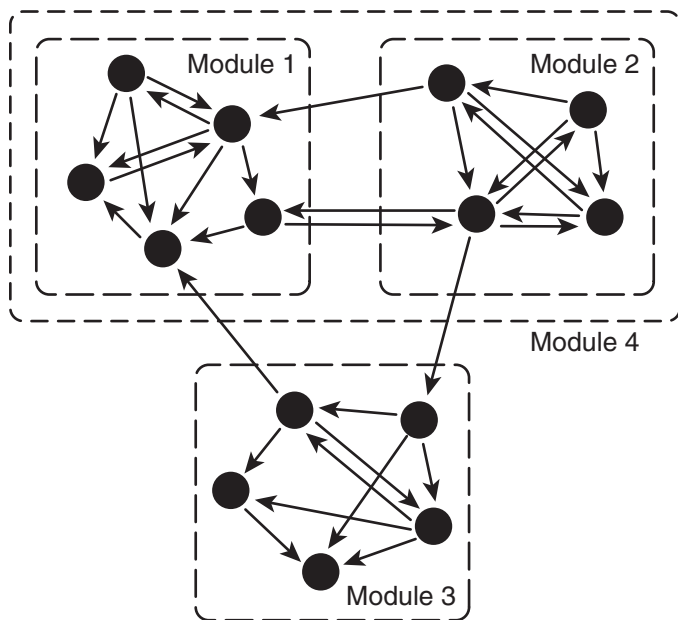


FIGURE 11-2. Modules and developmental interactions. A module is a set of traits (circles) that is rendered internally coherent by multiple interactions (arrows) among the constituent traits and relatively independent from other modules because there are fewer or weaker interactions between modules. Modularity is hierarchical in that modules at one level can be the traits that make up a module at a higher level of organization.

occurring later in ontogeny, for instance, bone remodeling (Herring, 1993; Enlow and Hans, 1996), can potentially have major effects on the patterns of modularity.

Integration manifests itself as the covariation among morphological traits. As such it can be analyzed statistically using morphometric data. Different methods have been used since the inception of this approach, including the analysis of correlations among distance measurements (e.g., Olson and Miller, 1958; Cheverud, 1982; Leamy and Atchley, 1984; Zelditch, 1987; Cheverud, 1995) or among the positions of morphological landmark points (Klingenberg and Zaklan, 2000; Klingenberg *et al.*, 2001a; Bookstein *et al.*, 2003; Klingenberg *et al.*, 2003). The patterns of covariation among traits can provide important information about modularity: Modules are expected to be sets of traits that are highly integrated internally and relatively independent among each other, whereas structures that are completely integrated will show high degrees of morphological covariation in all their parts.

III. DEVELOPMENTAL ORIGINS OF COVARIATION AMONG TRAITS

Different mechanisms can contribute to covariation among traits, including genetic and environmental factors acting on the traits through various mechanisms. To understand the influence of integration and modularity on evolvability and constraints, it is important to understand the developmental basis by which the covariation comes about and how this question can be addressed by empirical studies (Klingenberg, 2003a, 2004).

Two conditions are required for covariation of two or more traits: There must be variation, and there must be a mechanism creating an association between the traits so that the variation affects them jointly. Depending on the particular circumstances, the source of variation and the mechanism causing the association between traits may be separate processes, or the source of variation itself may be responsible for the association. The developmental basis of the mechanisms that create this association can have a substantial influence on the evolutionary effects of integration and modularity. It is therefore important to examine these mechanisms in some detail.

There are two main types of mechanisms that give rise to covariation of morphological traits: direct developmental interaction between the developmental pathways that produce the traits (Figure 11-3A, B) and parallel variation in pathways that are separate from each other (Figure 11-3C). In the first type, there is a direct interaction between two pathways that creates the association between them by passing on the variation to both pathways. The variation can be passed down from earlier (“upstream”) steps in the pathway and therefore need not be associated with the mechanism of interaction that generates the association

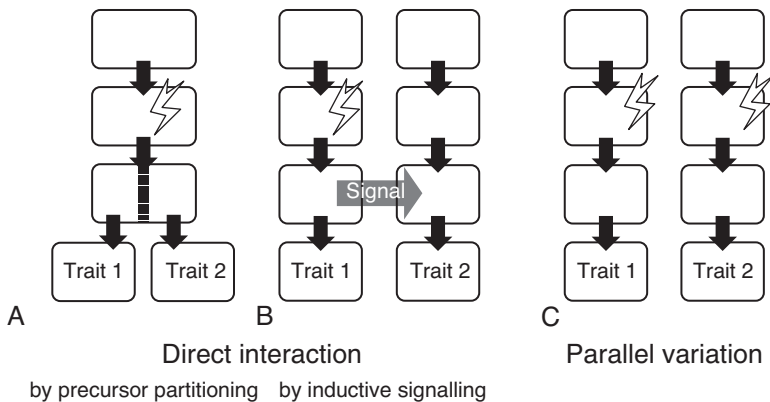


FIGURE 11-3. Mechanisms that generate covariation between traits. The diagrams depict developmental pathways that produce traits in several steps. Variation in a pathway (lightning bolts) can be transmitted through the pathways and thereby affects the respective traits. In (A) and (B), this variation is shared between traits because a direct interaction of the pathways transmits the variation directly. This interaction can be the bifurcation of a single pathway into two pathways by partitioning of a developmental precursor (A), a signal going from one pathway to the other (B), or other forms of developmental interaction. In (C), the two pathways are separate and there is no direct interaction between them. In this case, covariation is entirely the result of the simultaneous effect of the same source of variation on each pathway separately (separate lightning bolts in the two pathways).

between the traits. In contrast, for parallel variation of pathways, two different pathways are affected by a factor causing variation in both of them simultaneously, and the factor responsible for the association between traits is therefore the source of variation itself.

Direct interaction between developmental pathways can occur in a variety of ways. One example is the division of a precursor tissue into two distinct populations, which may correspond to distinct anatomic parts (Figure 11-3A). Variation that arises in the common upstream part of the pathway (lightning bolt in Figure 11-3A) is passed down through the partitioning step to both parts simultaneously, creating a positive covariance between them (Riska, 1986). In contrast, variability in the ratio of partitioning itself will produce a negative association between the two traits (Riska, 1986), as they will compete directly for the precursor tissue (e.g., Nijhout and Emlen, 1998). Another type of direct interaction is inductive signaling from one pathway or tissue to another, which can transmit variation from the pathway emitting the signal so that it simultaneously affects both pathways (Figure 11-3B). Signaling interactions are of fundamental importance for many patterning processes in development (e.g., Francis-West *et al.*, 1998; Gurdon and Bourillot, 2001), and accordingly, there is considerable opportunity for this mechanism of integration. A hallmark of covariation caused by direct interaction of developmental pathways is that variation arising within the pathways themselves

(lightning bolts in Figure 11-3A, B) can be transmitted to other pathways. This intrinsic developmental variation is therefore a source of covariation between traits.

In contrast, the mechanism of parallel variation in separate pathways relies on the simultaneous action of an outside factor (lightning bolts in Figure 11-3C) on multiple pathways to generate covariation among traits. For this type of mechanism, therefore, the source of variation itself also provides the basis for the association between traits. The variation can come from environmental factors that have effects on several developmental pathways simultaneously, for instance, temperature differences or nutritional factors. Alternatively, allelic variation of genes that are involved in both pathways can generate parallel variation, even if they are expressed at different anatomic locations or different stages of development. An example would be the *Distal-less* (*Dll*) gene, which is involved both in the development of the distal parts of the legs and antennae and of the color patterns on the wings of butterflies (Panganiban *et al.*, 1994; Brakefield *et al.*, 1996; Beldade *et al.*, 2002a). If alleles of *Dll* differ in their developmental activities in these different contexts jointly, then variation at the *Dll* locus will have simultaneous effects on multiple traits and can generate covariation between them. For covariation by parallel variation of separate pathways, the origin of variation lies outside the individuals, for example, if individuals experience differences in environmental conditions or carry different alleles of a gene. The association between traits arises because this variation affects the development of multiple traits in each individual simultaneously. In neither of these cases is there a direct interaction between the pathways that produce the traits. Variation arising within any pathway only affects downstream steps within that pathway itself but has no effects on other pathways. Therefore, it does not produce covariation between traits.

A possibility to distinguish between the two main origins of covariation among traits is therefore to control rigorously for genetic and environmental variation. Eliminating these components of variation will also eliminate covariation by parallel variation of separate pathways. Any covariation between traits, under these conditions, will therefore be from direct connections between pathways.

A particularly convenient way to achieve control over external variation is to focus on fluctuating asymmetry, the small random differences between bilateral structures of the left and right body sides (Palmer and Strobeck, 1986; Møller and Swaddle, 1997). The two body sides share the same genome and nearly the same environment, at least for most mobile organisms, and focusing on asymmetries therefore provides a means to minimize external variation. The differences between left and right sides are caused by random fluctuations in developmental processes, for example, from relatively small numbers of certain molecules involved in transcriptional regulation or signaling (McAdams and Arkin, 1999; Klingenberg, 2003b). Because this variation is random, a systematic association that is manifest statistically as a correlation between the asymmetries of traits can only occur if the effects of the perturbations are transmitted from a source of variation to the traits,

which in turn requires a direct interaction between the respective developmental pathways. Moreover, because this spontaneous variation is intrinsic to the developmental pathways, it can only be transmitted between pathways by direct interaction. Accordingly, covariation of fluctuating asymmetries among traits is therefore exclusively the result of direct interactions between pathways (Klingenberg, 2003a). A number of empirical studies have used this method to examine the developmental basis of morphological integration and modularity (Klingenberg and Zaklan, 2000; Klingenberg *et al.*, 2001a, 2003).

The two sources of covariation are not mutually exclusive but can operate side by side. Simultaneous external influences can cause parallel variation in pathways that are also linked by a direct developmental interaction. Still, the analysis of covariation in the asymmetries of different traits will indicate the contribution of direct interaction to the total trait covariation.

IV. DEVELOPMENTAL INTERACTIONS AND PLEIOTROPY

The different origins of morphological covariation apply to broad classes of variation, and in particular, they also apply to variation that results from allelic differences that are involved in the developmental pathways. As a result, the respective loci have pleiotropic effects on multiple traits.

The mechanisms that produce pleiotropy have been the subject of study throughout the history of genetics, and the explanations used to account for it have reflected the changing concepts of developmental genetics (e.g., Grüneberg, 1938; Hadorn, 1945; Pyritz, 1989; Hodgkin, 1998; Nadeau *et al.*, 2003). Different types of pleiotropy have been distinguished, which partly match the current distinction of sources of morphological covariation. Grüneberg (1938) defined “genuine” pleiotropy as an ideal case in which a gene produces its effect on different traits by distinct mechanisms, but he expressed doubt about its existence. He opposed this mode to the more common “spurious” pleiotropy, in which the gene affects multiple characters by the same mechanism or where intermediate causes are involved. Similarly, Hadorn (1945, p. 91) distinguished “primary” and “secondary” pleiotropy. Primary pleiotropy is the direct result of the constitution of the cells that are the precursors of the traits, whereas secondary pleiotropy is generated by the transmission of effects from other cells to the separate populations of progenitor cells of different traits. In this distinction, primary pleiotropy closely corresponds to parallel variation of separate developmental pathways resulting from simultaneous allelic effects, and secondary pleiotropy results from direct interaction of the pathways that generate the traits. A much more elaborate classification of pleiotropy, subdividing primary pleiotropy into several types according to the biochemical modes by which parallel effects in

separate pathways come about, has been advanced more recently (Hodgkin, 1998). Here, I will concentrate on the two main categories.

Pleiotropy by direct interactions of developmental pathways has implications for the phenotypic effects of the respective loci. Accordingly, the mapping of functional and causal relationships in the developmental networks, which has been pursued since the inception of studies of pleiotropy (Grüneberg, 1938; Pyeritz, 1989), remains an activity that is central to this subject (Davidson *et al.*, 2003; Nadeau *et al.*, 2003). The particular nature of the connection between pathways will impart a specific pattern on the effects of multiple genes that are upstream of the connection. Variation is transmitted along developmental pathways in a manner specific to each developmental step rather than the source from which it originated. For instance, increases of several different activating transcription factors or decreases of inhibiting factors might all lead to increased expression of a gene, and in both cases, the response will be a greater concentration of that gene's product. Accordingly, each pathway "lumps together" the variation from the upstream steps so that the individual inputs may not be identifiable. The patterns of covariation among traits are generated through the interactions of different pathways and will be shared by those loci that contribute to the variation at the step where the interaction takes place and upstream of it. Because variation may be attenuated or amplified as it is transmitted through a pathway, and because each pathway may interact with several others at different steps, different loci may contribute differently to the interactions at different steps. Therefore, if a pathway interacts with several other pathways at various steps, the patterns of phenotypic effects may differ among groups of loci depending on the steps in which they participate. Nevertheless, there will be similarities between the effects of the loci that are active in the same steps.

In contrast, pleiotropy by parallel variation of separate pathways stems from allelic differences that jointly affect two or more developmental pathways in which the respective loci are active. The activity of a locus in multiple developmental contexts can come about by various mechanisms (Pyeritz, 1989; Hodgkin, 1998), but it is likely that the modular organization of *cis*-regulatory elements and the history of cooption of genes to new functions facilitate this multiple deployment of genes (e.g., Davidson, 2001; Wilkins, 2002; Levine and Tjian, 2003). Many genes that have important roles in development are expressed in different locations and at different stages of development (Carroll *et al.*, 2001; Davidson, 2001; Wilkins, 2002). For example, the *Distal-less* gene in butterflies is involved in generating the distal parts of the legs and antennae and also the colored eyespots on the wings (Carroll *et al.*, 1994; Panganiban *et al.*, 1994; Beldade *et al.*, 2002a). If alleles at such a locus differ among each other in their activity in different contexts jointly, they can cause pleiotropy by parallel variation of pathways. Examples of such effects are abundant and include the numerous syndromes caused by single mutations known in human medical genetics (e.g., Pyeritz, 1989; Jabs, 2002).

For pleiotropy of this kind, each locus provides not only the source of variation by differences in the developmental activity between alleles, but through the joint activity in two or more pathways, the gene is also directly responsible for its contribution to the covariation between the traits derived from the pathways. Accordingly, the patterns of covariance among traits can be as diverse as the loci exhibiting pleiotropy of this kind, and no grouping of loci by their patterns of phenotypic covariation can be expected.

In summary, the difference between these two mechanisms of generating pleiotropy is that direct interactions between pathways establish groups of loci that have similar patterns of trait covariation, whereas pleiotropy by parallel variation of pathways will result in phenotypic patterns that are individually different from locus to locus.

Empirical studies on the role of the two processes in generating pleiotropy of single loci are technically difficult and have only just begun to be undertaken. One possibility is to use multivariate approaches to mapping of quantitative trait loci (QTLs; Leamy *et al.*, 1999; Klingenberg *et al.*, 2001b; Workman *et al.*, 2002) and to analyze the patterns of QTL effects. These can then be related to patterns of trait covariation for fluctuating asymmetry, which provide a “standard” for the patterns generated by direct interaction of pathways (Klingenberg, 2003a, 2004). This approach has been taken for the study of shape variation in the mouse mandible, where both the patterns of covariation for QTL effects (Klingenberg *et al.*, 2004) and those for fluctuating asymmetry (Klingenberg *et al.*, 2003) show similar patterns of a partial separation between anterior and posterior modules. However, these studies are fraught with a number of inherent difficulties, such as weak statistical power with a limited number of QTLs (e.g., Flint and Mott, 2001), and further studies addressing these issues are needed.

V. EVOLUTION OF PLEIOTROPY AND DEVELOPMENTAL INTERACTIONS

The patterns of pleiotropy can undergo evolutionary change by changes in the developmental mechanisms that produce pleiotropy. The two main groups of mechanisms that produce covariation between traits differ in their evolutionary flexibility and in the implications of evolutionary changes for the developmental system itself. I will address these questions by considering the consequences of changes, for instance by mutation, that would alter the patterns of pleiotropy.

Evolution of the patterns of pleiotropy through direct interactions of pathways must occur by changes in the interactions themselves. For instance, the ratio of partitioning of a developmental precursor may change (Figure 11-4A), or there may be a change of an inductive signaling process from one pathway to another (Figure 11-4B). These changes may come about by new mutations of large effect,

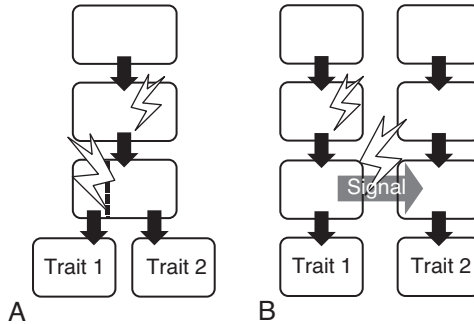


FIGURE 11-4. Genetic change in direct interactions of developmental pathways. (A) A change in the partitioning of a precursor (large lightning bolt) leads to a change in the patterns of covariation produced in response to variation transmitted from the “upstream part” of the pathway (small lightning bolt). For instance, this could be a change in the pleiotropic effects of a gene participating in the pathway. (B) Similarly, a change in the signaling interaction (large lightning bolt) may produce a change of the pattern of covariation in response to variation in the pathway (small lightning bolt).

or they can be based on the standing variation in a population, by changes in allele frequencies for loci that affect the interactions. The changes may be based on a variety of mechanisms. For instance, the partitioning of a cell population into different components may rely on the readout of morphogen gradients (Gurdon and Bourillot, 2001). Changes can affect the morphogen secretion, the mechanisms of morphogen transport, or the abundance of receptors on the responding cells and the intracellular signal transduction mechanisms that elicit their response. Similar cellular and molecular components may also be involved in changes in inductive signaling among pathways, strengthening or weakening the sensitivity of the target pathway to the signals from a source.

Any change of the interactions among developmental pathways will affect the patterns of pleiotropy of all loci that are upstream of the interaction as well as the integration of nongenetic components of variation. These changes thus affect the modular structure of the developmental system as a whole, and such a reorganization of the system is likely to have substantial effects on the phenotype. Examples of these effects can be found among major teratologies, which may be caused by the failure of specific developmental interactions (Alberch, 1989; Wilkie and Morriss-Kay, 2001; Cohen, 2002). Because of these serious consequences, changes in the connections among pathways will normally be selected against if the traits that depend on the respective developmental system are under stabilizing selection. There may even be selection for modifiers that stabilize the interactions among developmental pathways. Remarkable stability of the molecular mechanisms that set up or respond to morphogen gradients has been demonstrated empirically for embryonic patterning in *Drosophila* (e.g., Eldar *et al.*, 2002; Houchmandzadeh *et al.*, 2002) and may occur in different developmental contexts as well. As a result

of such robustness, the patterns of pleiotropy for multiple upstream loci are stabilized simultaneously.

An example of evolutionary changes in a signaling interaction and their far-reaching consequences is the loss of eyes in cave populations of the fish *Astyanax mexicanus* that have originated from surface-living forms on multiple occasions (Jeffery, 2001; Jeffery *et al.*, 2003). In cave fish, the lens cells undergo apoptosis, the eye primordia stop developing, degenerate, and are eventually covered by skin. A cave fish lens transplanted to a surface fish embryo undergoes apoptosis, whereas a surface lens transplanted to a surface fish stimulates the development of a complete eye (Yamamoto and Jeffery, 2000). Lens transplants also alter the growth of the orbital bones surrounding the eyes and the size of the olfactory pit, whereas other craniofacial differences between cave and surface fish appear to be independent of the eye (Yamamoto *et al.*, 2003). A change in signaling from the lens to other parts of the eye and adjacent structures therefore is responsible for much of the difference between the cave and surface forms. Given the dramatic effects of the change, it is plausible that this signaling interaction is under stabilizing selection in the surface populations.

The difference between cave and surface fish is visible much earlier in embryonic development as a difference in *Pax6* expression patterns (Jeffery, 2001). The restricted *Pax6* expression in cave fish, in turn, appears to result from increased signaling by *sonic hedgehog* (*Shh*) from the midline, which suppresses *Pax6* (Yamamoto *et al.*, 2001; Jeffery *et al.*, 2003). Injection of *Shh* mRNA into early embryos of surface fish results in a reduction of *Pax6* expression and eye regeneration similar to those in cave fish (Yamamoto *et al.*, 2001; Jeffery *et al.*, 2003). Therefore, the early change in *Shh* signaling appears to cause the later degeneration of the lens and loss of its signaling activity. There are therefore two direct developmental interactions involved, with the *Shh* signal from the midline upstream of the lens signal. There is indirect evidence for stabilizing selection on *Shh* activity, because both increases and decreases can cause malformations. Knock-outs of the *Shh* gene in mice have been shown to cause cyclopia (a single eye positioned in the midline; Chiang *et al.*, 1996) and mutations with reduced *Shh* activity have been shown to cause human holoprosencephaly (various degrees of underdevelopment of the midline of the brain and face, with cyclopia as an extreme; Nanni *et al.*, 1999; Schell-Apacik *et al.*, 2003). In contrast, experimental increase of *Shh* activity in craniofacial primordial of the chick produces an expansion of midline structures and may be related to hypertelorism in humans (enlarged distance between the eyes and overdevelopment of midline features; Hu and Helms, 1999). Because of the serious developmental consequences of both decreases and increases of *Shh* activity, it is reasonable to think that the signaling level is under stabilizing selection. With an ecological change such as the transition between surface and cave living for the fish *Astyanax*, this selective regimen may change (Jeffery, 2001; Yamamoto *et al.*, 2003). The result is that a whole suite of important changes, affecting

developmental processes and morphological traits, will take place as a response to a single genetic change in a crucial developmental interaction.

A very different set of conditions for the evolution of pleiotropic effects exists when pleiotropy originates by parallel variation in separate developmental pathways. Because the source of variation is itself the basis for the coupling of effects between traits, the pleiotropic effects of each locus are free to change independently of other loci. It is to be expected that *cis*-regulatory control of gene expression (e.g., Davidson, 2001; Levine and Tjian, 2003) is an important mechanism determining these effects and that many evolutionary changes of pleiotropic effects will result from the evolution of these control mechanisms (Wray *et al.*, 2003).

For simplicity, I will outline the changes of pleiotropic effects by parallel variation for a hypothetical gene with two separate *cis*-regulatory elements (promoters) that control expression of the gene in two different developmental pathways (Figure 11-5). If an allelic difference is located in the coding region of the gene and affects the developmental activity of the protein product, this difference will have an effect on both pathways simultaneously and will show pleiotropy of the resulting traits (Figure 11-5A). In contrast, an allelic difference that is located in one of the regulatory regions will only change the level of gene expression in the respective pathway but will leave the other pathway unaffected (Figure 11-5B, C). Accordingly, such allelic differences in single regulatory regions do not have pleiotropic effects on the resulting traits. This applies equally to differences resulting from new mutations and to differences between alternative “wild-type” alleles occurring in populations. New mutations or recombination events in the coding and regulatory regions of a gene can produce new patterns of joint effects on different traits. Because the regulation of each gene can evolve more or less independently, no particularly strong effects opposing evolutionary change are to be expected. Studies of real examples of genetic variation for gene regulatory sequences have found considerable variation and evolutionary potential (e.g., Rockman and Wray, 2002; Romano and Wray, 2003; Wray *et al.*, 2003). Therefore, it can be expected that pleiotropy by parallel variation will exhibit a considerable evolutionary flexibility.

In reality, the processes of gene expression contain additional complexities beyond the simplified description given here (Figure 11-5), which add more detail but do not change the principal conclusions drawn here (e.g., Davidson, 2001; Levine and Tjian, 2003). Not all allelic differences in the protein coding regions of a gene may produce pleiotropic effects, for instance, because alternative splicing of transcripts may restrict the effects of allelic differences to special developmental contexts and therefore to one pathway or another. These processes provide extra possibilities for the evolution of pleiotropy by parallel variation and therefore reinforce the conclusion drawn from the simplified model.

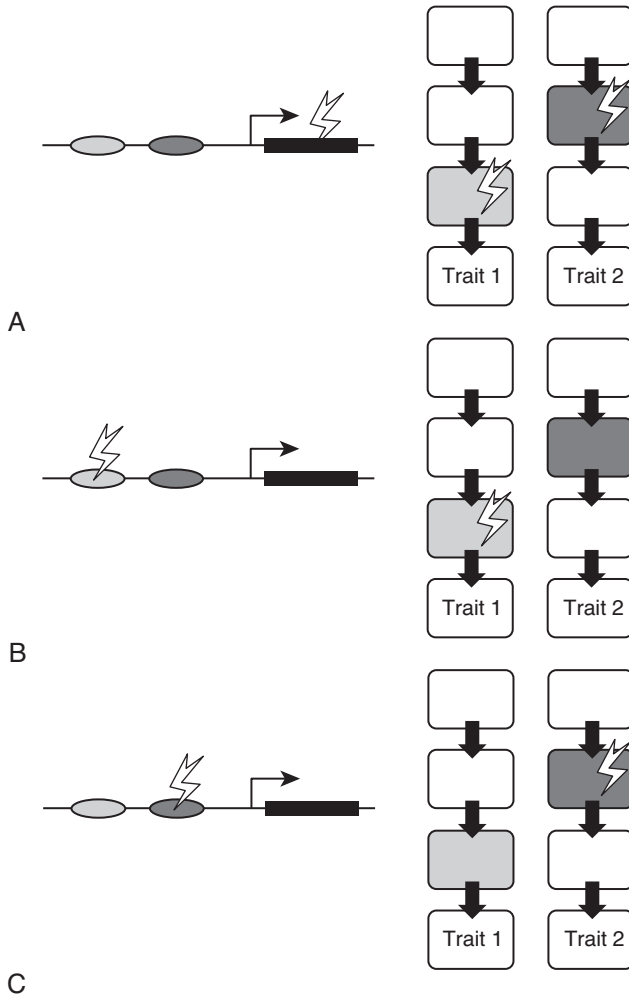


FIGURE 11-5. Mutation of regulatory and coding sequences of a gene and their consequences on pleiotropy of the gene by parallel variation of pathways. The gene has two regulatory modules (ellipses) that activate transcription in two different developmental contexts (corresponding shading of the regulatory module and the respective step in one of the pathways). (A) A mutation in the coding region of the gene. This mutation will have an effect on the gene product itself and will therefore affect both pathways simultaneously, thereby producing pleiotropy between the traits originating from the pathways (unless there are complicating factors such as alternative splicing, etc.). (B) Mutation is one of the regulatory modules (light gray shading). The mutation produces an effect only in one of the pathways. Therefore only one trait is affected, and the mutation does not have a pleiotropic effect. (C) The mutation is in the other regulatory module (dark gray), and the effect is just the reverse of the situation in (B). There is no pleiotropy either.

VI. MODULARITY OF PLEIOTROPIC EFFECTS: INHERENT IN DEVELOPMENTAL SYSTEMS OR EVOLVED PROPERTY?

The preceding discussion of pleiotropy has focused on its developmental origins, and likewise, modularity has been defined exclusively in terms of direct developmental interactions. This perspective is viewing modularity as an outcome of the developmental system, which is an intrinsic feature of the organism that can produce patterns of variation and constraints (Seilacher, 1974; Gould and Lewontin, 1979; Alberch, 1989; Gould, 2002).

This explanation for the origin of modularity of genetic variation is an alternative to the perspective advanced by Wagner and Altenberg (1996) and Wagner (1996), which is related to ideas published by Cheverud (1982, 1984), Riedl (1975), and Olson and Miller (1958). This explanation is based on the assumption that selection favors integration within complexes of traits that serve a particular function. For instance, integrated variation of upper and lower jaws will be necessary to ensure proper occlusion and therefore to support functions such as biting and chewing effectively. Accordingly, selection is expected to favor pleiotropy among those traits that belong to the same functional units. Because different functions can pose different adaptive demands, the same reasoning suggests that pleiotropic effects among traits that serve different functions would be disfavored. Selection will tend, on the one hand, to extend the pleiotropic effects of genes to the sets of traits serving particular functions, and on the other hand, to break up pleiotropic complexes of traits that are involved in different functions (integration and parcelation in the terminology of Wagner and Altenberg, 1996). As a result of this adaptive process, separate sets of loci will have effects on the sets of traits associated with different functions. The genetic modularity will match the subdivision of morphological structures into functional units. The genetic modules are distinct sets of loci, each internally connected by a network of pleiotropic effects, which will map directly to functional modules, sets of traits related by shared functions (Wagner and Altenberg, 1996). The crucial point of this view is that modularity is the outcome of selection for variation that can accommodate groups of traits serving different functions.

This theory assumes that the sets of traits affected by pleiotropy can evolve. This means that the loci accounting for the genetic variation of the traits possess alleles that differ in the distribution of pleiotropic effects. This assumption is the standard in theoretical quantitative genetics, which like many other areas of genetics, is primarily based on the differential gene concept. This concept defines genes as units of genomic change associated with phenotypic change without specifically considering the processes involved (Gilbert, 2000; Schwartz, 2000). Therefore, the primary aim is to construct a connection between allelic variation and the associated phenotypic differences. The resulting “genotype–phenotype map” (Wagner and

Altenberg, 1996) is an abstract mapping between genes and phenotypic characters that does not take into account the developmental mechanisms by which genes exert their effects on the phenotype (diagrams represent the mapping by straight arrows from genes to characters; e.g., Wagner, 1996; Wagner and Altenberg, 1996). This may be surprising given the prominent place development takes in the discussion of these models (Cheverud, 1982, 1984, 1996; Wagner, 1996; Wagner and Altenberg, 1996). This point is of far more than just symbolic importance, because the developmental system that mediates between genes and phenotypes will determine the extent to which pleiotropic effects are variable among alternative alleles and new mutations of the loci that affect the traits.

If pleiotropy originates by parallel variation of separate developmental pathways, there should be plentiful variation in the patterns of pleiotropy and ample opportunity for evolutionary change (Figure 11-5). In contrast, if pleiotropy mainly originates from direct interaction of developmental pathways, there may not be much variation in the pleiotropic patterns among alleles, because the interaction imparts similar patterns of pleiotropy to all upstream loci. Even after a change of the interaction itself, variation in pleiotropic patterns may not increase because the change of the interaction may simply yield a switch to a new pattern of pleiotropy that might still apply to all upstream loci simultaneously. Pleiotropy by direct interaction of developmental pathways therefore produces conditions that are less favorable for the adaptive evolution of pleiotropy at individual loci. The developmental origin of pleiotropy therefore clearly matters for the evolution of modularity. This consideration on how pleiotropy can evolve, however, does not address the question of whether modularity is an evolved property.

The empirical evidence is indecisive. A considerable amount of work has been done on integration and modularity in the mouse mandible (Atchley and Hall, 1991). Studies of the overall genetic variation have produced evidence for a degree of subdivision into anterior and posterior modules (Atchley *et al.*, 1985; Cheverud *et al.*, 1991; Klingenberg and Leamy, 2001), consistent with differences in the functions and embryonic precursors of the different parts (Atchley and Hall, 1991; Atchley, 1993; Tomo *et al.*, 1997). Several studies have examined the pleiotropic effects of individual QTLs and found patterns consistent with a subdivision into two modules (Cheverud *et al.*, 1997; Mezey *et al.*, 2000; Ehrich *et al.*, 2003; Klingenberg *et al.*, 2004). However, the patterns of overall genetic variation and of individual QTL effects were consistent with the patterns of correlated fluctuating asymmetry that are indicative of direct developmental interactions (Leamy, 1993; Klingenberg *et al.*, 2003). This evidence is consistent both with the hypotheses of modularity as an adaptively evolved property and with the alternative that modularity is an automatic outcome of the developmental system. Distinguishing between these two hypotheses poses substantial challenges for empirical studies.

In many ways, the question of whether modularity has originated by adaptive evolution or as an automatic outcome of developmental systems parallels the

debate about the origin of dominance (Kacser and Burns, 1981; Orr, 1991; Porteous, 1996; Mayo and Bürger, 1997; Bourguet and Raymond, 1998; Bourguet, 1999; Omholt *et al.*, 2000). Just as is the case for dominance, it is clear that modularity is associated with most developmental systems and that it can evolve. The conditions are therefore met for both alternatives. To resolve the question of the relative importance of the two factors decisively, special experimental systems will be required. A possible approach is to use systems where one of the factors has been ruled out, as in the analysis of novel phenotypes such as mutants and teratologies, which are nonfunctional and therefore not the product of adaptation (Alberch, 1989; Dworkin *et al.*, 2001; Monteiro *et al.*, 2003). Another possibility is to study the evolution of systems where the functional and developmental units are clearly incongruent. Such studies have yet to be undertaken.

VII. FROM PLEIOTROPIC GENE EFFECTS TO G MATRICES

Regardless of the mechanisms that generate pleiotropic effects of single loci, a population's potential to respond to selection or to evolve by drift depends on the aggregate effect of all the loci affecting a set of traits. It is therefore important to evaluate the consequences of developmental changes for the overall quantitative genetic setup of phenotypic traits. To understand the consequences for evolvability and constraints of morphological traits, this section will examine how the effects of individual loci combine to the overall patterns of the genetic covariance matrix G and therefore the potential for evolutionary change (Lande, 1979; Roff, 1997; Steppan *et al.*, 2002). The modes by which pleiotropy is produced have different implications for the genetic covariance structure.

A direct interaction of pathways simultaneously imparts its pattern of pleiotropy to all the loci upstream in the pathway, from which it receives an input of variation. As a result, all these loci have more or less congruent patterns of pleiotropy, which they contribute to the overall pattern of genetic variation. After adding these effects from all loci, the interaction may therefore have a substantial influence on the structure of the total genetic variation, as it can be characterized by the G matrix. Because these patterns of genetic integration are also expected to coincide with the subdivision of morphological structures into developmental modules, the G matrix is expected to reflect this modular structure.

In contrast, the loci whose pleiotropy is based on parallel variation of separate pathways generate a diversity of different patterns, where each locus may have its own characteristic pleiotropic pattern. When the effects of all loci are combined, this diversity of different pleiotropic patterns will tend to "dilute" the effects of direct interaction. As a result, the patterns of overall genetic variation may only

coincide to some degree with the modules defined by direct developmental interaction, but no complete separation is to be expected (e.g., Klingenberg and Leamy, 2001; Klingenberg *et al.*, 2003).

In addition to these effects of pleiotropy, another contribution to the G matrix comes from loci that may not have pleiotropic effects at all, but jointly affect multiple traits because of linkage disequilibrium between them (Falconer and Mackay, 1996; Lynch and Walsh, 1998). Because linkage disequilibrium depends on the population structure, this contribution to the genetic covariances is expected to be highly variable over time. Moreover, the contribution of linkage disequilibrium to genetic covariance structure has no relationship to the modular structure of phenotypic traits. The relative importance of pleiotropy and linkage disequilibrium for the G matrix has rarely been investigated, but a study of genetic correlations among floral traits in wild radish suggested that pleiotropy was the primary factor (Conner, 2002).

VIII. G MATRICES, CONSTRAINTS, AND EVOLUTIONARY DYNAMICS

The components of genetic covariance from the different developmental origins differ substantially in their effects on evolutionary dynamics. How pleiotropy of individual loci can evolve has been discussed in the preceding text, and this section will expand on those arguments to explore evolutionary change of entire G matrices and its implications for the dynamics of evolution of the mean phenotype.

Because pleiotropy by parallel variation is specific to each locus, its effects on the G matrices will conform to the situation implied by the models of the evolution of genetic covariance structure, where each locus can have alleles with different pleiotropic patterns, which are subject to selection (e.g., Lande, 1980). Therefore, this contribution to genetic covariance structure will be evolutionarily malleable, and modularity can easily evolve to reflect the functional subdivision of morphological structures (Cheverud, 1982, 1996; Wagner and Altenberg, 1996). Because of the flexible nature of this source of pleiotropy, absolute or lasting genetic constraints are not expected to originate in this manner. These evolutionary changes will occur in a more or less gradual manner, depending on the magnitudes of effects of individual alleles. The influence of linkage disequilibrium on the genetic covariance structure is likely to be similar, but will be even more transient.

Direct developmental interactions not only differ from the other components of genetic covariance because they tend to shape the G matrix in a specific way corresponding to the developmental modularity, but they also have particular implications for evolutionary dynamics. As mentioned in the preceding text (see “Evolution of Pleiotropy and Developmental Interactions”), it is likely that

fundamental changes of the developmental architecture in a complex of traits are usually selected against and that direct interactions therefore provide a sort of buffering for the patterns of pleiotropy. There is little direct evidence on this subject, and empirical studies are clearly needed (even though they may be technically challenging). However, examples such as that of the *sonic hedgehog* gene, where mutations have serious adverse effects regardless of whether they reduce or increase signaling activity (Hu and Helms, 1999; Schell-Apacik *et al.*, 2003), provide indirect evidence for stabilizing selection. Moreover, there are indications for evolutionary conservation of the developmental function of gene regulatory systems even despite divergence in regulatory sequences, suggesting that the function of the whole systems is under stabilizing selection (e.g., Ludwig *et al.*, 2000; Romano and Wray, 2003). Overall, it is likely that direct developmental interactions are evolutionarily conservative.

This evolutionary conservatism of direct developmental interactions also tends to render the resulting patterns of pleiotropy resistant to change. Moreover, because all loci upstream of a direct interaction will obtain the same pleiotropic pattern from it, these stable patterns may make a substantial contribution to the total genetic variation. As a result, the **G** matrix will be fairly stable over evolutionary time, which may be manifest in comparative genetic studies as a similarity of **G** matrices of related populations or even species (Kohn and Atchley, 1988; Roff, 1997, 2000; Steppan *et al.*, 2002). Any genetic constraints may have a sustained influence on the evolutionary trajectories of populations.

Once changes in direct developmental interactions signaling mechanisms do occur, however, the consequences can be momentous. The change in *sonic hedgehog* that appears to account for the loss of eyes in cave fish and its manifold effects on craniofacial morphology is a clear example of this (Jeffery, 2001; Yamamoto *et al.*, 2001, 2003). Because the direct interactions influence the effects of all genes that act upstream of the interaction, a change in the interaction will trigger a change in the pleiotropic effects of all these loci. Such a concerted change of the pleiotropic patterns of multiple loci can have a substantial effect on the total genetic covariation among traits, which results from the combined effects of all loci in the whole system. Hence, a single change of developmental interactions can precipitate a fundamental change not only of the average morphology, but also of the standing stock and organization of genetic variation in the population and thus may substantially alter its potential to respond to selection.

As a result of a change in a direct developmental interaction between pathways, the **G** matrix may change considerably and, along with it, the potential for evolutionary change. Reorganization of the developmental system is therefore likely to be accompanied by a reorganization of the genetic constraints and evolvability of the structure. Because a single change is potentially sufficient to produce this reorganization, changes in the **G** matrix may be rapid. If reorganization is favored by selection and progresses through the population as a selective sweep, then the

change of the G matrix may appear to be instantaneous on an evolutionary time scale.

If that is the case, then the evolution of patterns of pleiotropy may proceed in a punctuated manner in which extended periods of stabilizing selection and no change of the pleiotropic relationships are interspersed with short episodes of developmental reorganization of the modular structure of developmental systems that may coincide with dramatic changes in the genetic and phenotypic covariance structure. Phases of strong directional selection on the mean phenotype might override the effects of stabilizing selection and favor the reorganization of the developmental interactions among developmental pathways. Such episodes of change of the covariance structure would remove genetic constraints of the evolution of the population average phenotype and release new phenotypic variation. The molding of phenotypic variation by developmental interactions between pathways therefore can act in a manner analogous to the evolutionary “capacitance” by some mechanisms of phenotypic buffering (Rutherford and Lindquist, 1998; de Visser *et al.*, 2003; Rutherford, 2003). The release of variation in response to the developmental reorganization may be a factor contributing to classical punctuated evolution as it has been characterized from the fossil record (reviewed by Gould, 2002).

IX. PERSPECTIVE: DEVELOPMENTAL PROCESSES AND EVOLUTIONARY CONSTRAINTS

This discussion of the developmental origins of morphological covariation and modularity has substantial implications for the interpretation of constraints in evolution. A number of authors have discussed whether modularity enhances evolvability or is necessary for it (Cheverud, 1996; Wagner, 1996; Wagner and Altenberg, 1996; Hansen, 2003). My approach here is slightly different from these discussions, as I consider the ways in which modularity and pleiotropy come about in developmental systems. I have outlined in the preceding text that the mechanisms responsible for generating pleiotropy differ considerably in their evolutionary behavior, both in terms of the likelihood of change and, once a change occurs, in how profound its effects will be. The primary conclusion that emerges from this line of reasoning is that the developmental origin of the covariation among traits is a factor of prime importance for evolutionary quantitative genetics.

Patterns of pleiotropy that originate by the direct interaction of developmental pathways are likely to be evolutionarily conservative, but once a change occurs, it will simultaneously affect multiple upstream loci in a coordinated manner and will therefore have a profound effect on the G matrix. As a result of this change in the modular structure of the system, the patterns of genetic constraints will also change, and the potential for response to selection will be altered as well. A single change of the developmental system may thus release an avalanche of changes in

the evolutionary potential of the traits, which may manifest itself as a punctuated change in the evolutionary behavior of the evolving lineage.

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