Integration, modules, and development: molecules to morphology to evolution

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Introduction

Living organisms are intricately organized developmental systems, which at the same time are very flexible but also highly robust. They are flexible to respond to environmental conditions by changing developmental processes and the resulting phenotype accordingly, but they are also robust in that all these developmental changes in different parts are coordinated and the end result is an integrated, functional organism. Similarly, there is considerable flexibility for evolutionary change of specific parts, while robustness of the overall body plan ensures continued integration of multiple organismal functions.

Research in recent years has identified the modular architecture of organisms as one of the major principles that underlies this simultaneous flexibility and robustness (e.g., Raff 1996; Kirschner and Gerhart 1998; von Dassow and Munro 1999; Bolker 2000; Winther 2001). Modules are units that are made internally coherent by manifold interactions of their parts, but are relatively autonomous from other such units with which they are connected by fewer or weaker interactions (Fig. 1). Modules are therefore "individualized" to some extent and can be delimited from their surroundings. They are units that can function in different contexts and can undergo developmental and evolutionary change separately. Modular organization has been found at many levels of organization, from molecular structure of individual genes to the body plans of whole organisms. At the molecular level, *cis*-regulatory sequences of single genes are subdivided into distinct modules that control expression of the

gene in different locations or at different times in development (Yuh et al. 1998; Davidson 2001). Modularity is also found in gene regulatory networks, where the interactions among genes tend to be concentrated in particular "clusters" that are stable in themselves, and where such modules can be flexibly deployed in different developmental contexts (von Dassow et al. 2000; Wilkins 2002, pp. 348–350). The most apparent manifestation of modularity, of course, is in the structural parts that make up the bodies of organisms, where modules can originate as developmentally distinct parts or perform different functions (Cheverud et al. 1997; Klingenberg et al. 2001). These examples show how different the domains are to which the principle of modularity can be applied. Modules can be tangible material units as in the examples of morphological parts or of *cis*-regulatory modules that are specific stretches of DNA sequence, or they can be abstract as in the example of gene regulatory networks, where modularity resides in the regulatory relationships among genes. In modular systems at all levels, however, the primary criterion for identifying modules is the strong internal coherence and connectivity of modules coupled with their relative independence from other parts of the system (for additional discussion, see Bolker 2000).

Phenotypic studies address modularity primarily at the morphological level. The body parts of organisms behave as modules because they are internally coherent and show some degree of mutual autonomy corresponding to their developmental origins and functions (Cheverud 1996; Wagner 1996). Developmental biologists have long considered modules of this kind under the concepts of morphogenetic fields or embryonic fields (e.g., Davidson 1993; Gilbert et al. 1996; Wilkins 2002, p. 255–258). These modules are internally coherent due to signaling interactions that are part of the patterning processes that generated the structure. In the resulting body parts this coherence is manifest as morphological integration (e.g., Olson and Miller 1958; Cheverud 1996). Therefore, developmental integration and modularity are amenable to quantitative study with morphometric methods (Pimentel 1979; Bookstein 1991; Dryden and Mardia 1998).

In this chapter, I review the developmental origins of integration and modularity from molecular mechanisms to their morphological manifestation. Consideration of these issues reveals that morphological variation originating from different sources intrinsic or extrinsic

to the organism can be analyzed to infer the developmental origins of integration and to delimit the spatial extent of morphological modules. I describe this morphometric approach for identifying developmental modules and briefly review the few available case studies. I also examine the implications of this developmental perspective on morphological integration for evolutionary quantitative genetics, where it can shed new light on the evolution of pleiotropy and genetic covariances.

Modularity and integration by intercellular signals

Modules are units within a developmental system that are defined by their internal coherence and relative independence from other parts of the system. They are made internally coherent by manifold interactions among their component parts, and the nature of those interactions is therefore a defining property of the modules themselves. Different kinds of modules are based on different kinds of interactions, but they are recognizable because there are numerous and strong interactions within modules and fewer or weaker interactions between a module and the rest of the system.

In a morphological context, developmental modules are spatially delimited domains of developing organisms within which signaling interactions take place that organize patterning and morphogenesis of the resulting adult structures. The interactions that give coherence to these modules must therefore act over the spatial scale of the module. Probably the most widespread mechanism for such interactions over a distance is signaling via morphogens (Neumann and Cohen 1997; Kerszberg 1999; Podos and Ferguson 1999; Gurdon and Bourillot 2001). The signaling molecules can be proteins, for instance, of the FGF, Hedgehog, Wnt, or TGF-β families, or other molecules such as retinoic acid (e.g., Begemann and Meyer 2001). Spatial patterning by morphogen gradients is a process consisting of two main steps: the establishment of the gradient and its interpretation by cells (Kerszberg 1999; Gurdon and Bourillot 2001).

Morphogen molecules are secreted by some cells and diffuse or are transported to others that may be several cell diameters away. The distances depend on the specific signal molecule, as there are short-range as well as long-range morphogens. Transport can occur via

"bucket brigades" of membrane-bound receptor molecules that can carry signaling molecules along the cell surface and from one cell to another, but relay mechanisms involving sequential uptake and re-release by cells have also been shown (Kerszberg 1999; Gurdon and Bourillot 2001). Morphogen transport, and therefore the shape of the gradient, can be influenced by the binding to receptors and interactions with antagonistic proteins, as well as degradation of the morphogen. As a result, many factors can at least potentially influence gradient shape (e.g., Entchev et al. 2000; Teleman and Cohen 2000), and there may also be ample opportunity for evolutionary changes.

Interpretation of morphogen concentration by cells occurs through cellular signal transduction pathways that are activated when morphogen molecules bind to receptors on the cell surface. Because the response to signaling is usually a change of the cell's transcriptional activity, the signal is transmitted from the activated receptors at the cell surface to the nucleus by signal transduction molecules. At least in the particularly well-studied case of activin signaling in *Xenopus* blastula cells, it has been shown that the absolute number of occupied receptors, and not the ratio of occupied to unoccupied receptors on the cell surface, determines the response of the cell (Dyson and Gurdon 1998), and that the transduction system operates linearly, that is, a threefold difference in the number of occupied receptors translates into a threefold difference in activated cellular transduction molecules (Shimizu and Gurdon 1999). The transduction proteins can interact with *cis*-regulatory elements of downstream genes and activate or repress their transcription. To the extent that cells are homogeneous in their interpretation of morphogen concentrations, morphogens will have a coordinating effect and integrate variation across the domain of signaling, thereby promoting the coherence of the module.

A highly simplified model of genetic control for a morphogen gradient and threshold response, when the phenotypic outcome was analyzed with the methods of quantitative genetics, produced complex outcomes including additive genetic variation, dominance, and epistasis among the components of the model (Nijhout and Paulsen 1997; Klingenberg and Nijhout 1999; Gilchrist and Nijhout 2001). A more realistic model including details of transcriptional control of a target gene by the concentration of a transcription factor yielded

similarly complex results (Gibson 1996). Given that many gene products are involved in setting up and interpreting morphogen gradients, it is clear that these systems offer a substantial potential for evolutionary change in signaling. Such evolutionary flexibility of signaling processes also provides the potential for changes in the spatial extent, patterning, and integration within developmental modules.

Morphogenetic fields

Developmental modules that are spatially defined units giving rise to specific body parts have been discussed in developmental biology in relation to the classical concept of morphogenetic fields (e.g., Gilbert et al. 1996). Morphogenetic fields (also termed secondary embryonic fields) are embryonic regions that are precursors of specific parts of the developing organism, which, once they have been established, have considerable autonomy from the development of other parts of the embryo (e.g., Wilkins 2002, pp. 255–258). This concept has recently been refined in the light of new information on the molecular mechanisms that establish and delimit the fields (Davidson 1993; Gilbert et al. 1996; Carroll et al. 2001; Davidson 2001, ch. 4; Wilkins 2002, pp. 302–305). A critical factor for the initial establishment of fields is intercellular signaling, in which cells that receive the initiating signal are set apart from neighboring cells to organize the prospective module. The distinctness of the field is usually assured by the expression of one or more transcription factors that act as field-specific selector genes (Carroll et al. 2001, pp. 26–28) and commit the cells to fates specific to the prospective body part. Once a field is specified, further signaling steps are activated, which mediate the patterning processes leading to further subdivision and specification within the field.

The cells within a morphogenetic field are not necessarily homogeneous, but there may be internal boundaries delimiting cell populations with different properties. For instance, the wing imaginal disc of *Drosophila* is divided into compartments, which are distinct cell lineages because cells normally do not cross the boundary to move from one compartment into another (Dahmann and Basler 1999; Irvine and Rauskolb 2001; Held 2002). Moreover, the compartments are also characterized by the expression of specific selector genes; for

instance, the posterior compartments of *Drosophila* imaginal discs express *engrailed*. The compartment boundaries are not just inert division lines separating distinct populations of cells, but they are themselves active signaling centers. For instance, in the *Drosophila* wing, perpendicular morphogen gradients of the Decapentaplegic and Wingless emanate from the anterior–posterior and dorsal–ventral compartment boundaries and set up a coordinate system of positional values throughout the imaginal disc (Lawrence and Struhl 1996). These signals have a double function. On the one hand, through the different expression patterns of target genes that differ in the concentrations required for transcriptional activation, the morphogen gradients define the further subdivision of the field into domains corresponding to specific portions of the final body part (e.g., Lecuit et al. 1996; Nellen et al. 1996; Held 2002). On the other hand, because the signals are transported to both sides of the respective compartment boundary, they are contributing to integration across the compartments.

The partitioning of the field into sub-domains creates new boundaries where populations of cells expressing different regulatory genes are juxtaposed to each other. These new boundaries can in turn be the origin of signaling through morphogens. Through sequential rounds of intercellular signaling and division of transcription domains, the initial pattern of the morphogenetic field can be elaborated (Davidson 1993; 2001, ch. 4). Because this process usually proceeds while the field itself is growing, signals that travel over a constant distance, as measured in cell diameters, will act at a successively smaller scale relative to the field as a whole. To make this stepwise elaboration of preexisting pattern elements more intuitive, Coen (1999, p. 131–143) has used the metaphor of an artist painting on an imaginary canvas that is expanding while the strokes of the paintbrush always have the same width — at first, the coarse outlines of the overall composition are laid out, whereas the later brush strokes add successively finer details.

The iterative patterning through successive rounds of signaling and establishment of transcription domains specifies the overall topology and pattern elements of the body part that will arise from the morphogenetic field. This specification of the prospective structure is by a combinatorial code of selector genes, whose transcription domains will overlap to various degrees, depending on the sequence of subdivisions. The organization of patterning

processes is therefore hierarchical, where overall integration is expected to result from the early signaling steps with morphogen gradients extending across the entire field, but where later patterning steps would generate only local integration within progressively finer sub-domains.

The regional code of selector genes in the morphogenetic field influences the patterning of the prospective structure by locally variable rates of cell proliferation and directional alignment of new cells (e.g., Resino et al. 2002). This pre-pattern is translated into the geometry of the final structure by differentiation of tissues and by morphogenetic movements of parts, for instance, deformations such as stretching, folding, and distal outgrowth. These processes do not all need to reflect the hierarchical fashion in which the domains for pattern elements originally were laid down in the field, and may even obscure some of the original localized structure by overall deformations that force different parts of the field to fit together. On the whole, these late morphogenetic events are not nearly as well understood as the early patterning processes, but they clearly have the potential to influence patterns of integration of morphological structures decisively.

Morphological integration

Integration resulting from developmental interactions can be studied by analysis of covariation among the parts of the fully formed structure. However, developmental connection is not the only cause of covariation, because genetic and environmental factors also may contribute to simultaneous variation of multiple parts. It is therefore helpful to examine briefly how covariation between morphological traits can arise (see also Klingenberg 2002a). Covariation is the regular association of variation between different traits. Therefore, if one trait deviates in a particular way from its average value, there is an expectation that a different trait will also deviate from its average in a specific direction. What is required for covariation is a source of variation and a mechanism that generates a regular association between the traits. The source of variation may be linked to the mechanism that generates the association, but this is by no means necessary. Associations between traits are generated primarily in two different manners: by direct connections

between the developmental pathways that produce the traits or by parallel variation of separate pathways that respond to the same extrinsic factors (Fig. 2; for a detailed discussion of the concept of developmental pathways, see Wilkins 2002, ch. 4).

In the preceding sections, I have discussed developmental signaling as a source of covariation, where signals originating from a restricted area such as a compartment boundary are transmitted through a much larger expanse of a morphogenetic field. Variation arising at the origin of the signal therefore is transmitted over a distance and can affect large parts of the developing structure simultaneously, generating systematic covariation. The patterning processes that subdivide the field into sub-domains, and therefore define the spatial organization of the prospective body part, also rely on these signals. Riska (1986) examined a series of models in which developmental precursors are partitioned into parts that give rise to different traits (Fig. 2A). Variation in growth before the fission will result in positive correlations between the resulting parts, whereas variation in the proportions allocated to the parts will generate negative associations. These elementary mechanisms are involved in complex developmental processes like the growth, partitioning, and migration of cell populations, for example in the neural crest, where they are critical determinants of patterning (e.g., Köntges and Lumsden 1996; Hall 1999). Therefore, processes like these can mold the associations among the resulting adult traits.

Signals from one pathway to another, often localized in distinct portions of the developmental field, are another mechanism that can generate covariation between the resulting traits (Fig. 2B). Such signaling also has been referred to as epigenetic control (Atchley and Hall 1991; Cowley and Atchley 1992; Hall 1999, ch. 7). Signals may even originate from adjoining structures outside the field itself, such as signaling from the endoderm to the cephalic neural crest and later between elements derived from them (Hall 1999; Couly et al. 2002), but still will cause covariation when the signal from one source has effects over an extended domain where it is received. Although these signaling mechanisms are likely to be the predominant source of interactions within modules that give rise to integration, there are also other processes that can result in direct transmission of variation between pathways.

All these effects are associations due to the direct developmental interactions among the developmental pathways that give rise to the parts concerned. These interactions can transmit variation originating in a single developmental pathway to multiple others, that is, variation from one source is transmitted to multiple pathways via the interactions among them, and can manifest itself as covariation among all the resulting traits.

The origin of the variation does not matter in this context: whatever the source of the variation in a given pathway, the variation will be transmitted to the other pathways if the variable step in the pathway precedes (is "upstream of") the developmental connection between pathways (Fig. 2A, B). If the variation is of genetic origin, its transmission to multiple traits generates pleiotropy (relational pleiotropy of Hadorn 1945; Pyeritz 1989; Wilkins 2002, p. 117–118). If the variation is environmentally induced, it will result in coordinated patterns of phenotypic plasticity. Even for random variation arising spontaneously within the developmental system itself (e.g., McAdams and Arkin 1999; Klingenberg 2002b), the connections of developmental pathways will result in patterned morphological variation.

There is another possible origin of covariation among traits, however, which is not based on direct connections between developmental pathways. This is the parallel variation of separate developmental pathways in response to extrinsic sources of variation that affect the pathways simultaneously (Fig. 2C). Joint variation of the morphological traits is produced by an outside factor that affects a step in each pathway and thereby elicits responses in all of them. The developmental effects of this extrinsic variation are transmitted in parallel along each of the developmental pathways, but not from one to another. Moreover, because no direct exchange between pathways occurs, the developmental precursors of the traits that covary in this way are not necessarily adjacent to each other — there is no need for any particular spatial relationship between them. Possible sources of variation include environmental factors such as temperature changes and nutrition. Allelic variation in genes that affect multiple developmental processes also can produce covariation in this manner, which is a form of pleiotropy, because the gene products are involved in multiple pathways that are otherwise independent (mosaic pleiotropy of Hadorn 1945; Wilkins 2002, p.

117–118). An example of such a gene is *Distal-less* in butterflies, where it is involved in the development of the distal parts of limbs as well as in the later specification of the colored eyespots on the wings (Carroll et al. 1994; Panganiban et al. 1994).

An important consequence of variation in separate pathways is that perturbations arising within the developmental pathway of one trait cannot be transmitted to other pathways and traits in this manner. To produce covariation by parallel variation, the source of variation must be extrinsic to the pathways themselves, and will usually be outside the developing organism as well. This is particularly clear for environmental variation, which affects the developing organism from outside. Genetic variation, although perhaps in a less obvious way, is also extrinsic to the developing organism, because it consists of differences in the genotypes among individuals that are already established at the zygote stage, but can of course affect the later development.

It is important to distinguish the two components of mechanisms that produce integration among morphological traits: on the one hand the source of variation, on the other hand the processes by which the variation is channeled into patterns of association between traits, that is, the processes that manifest the variation multiple traits simultaneously. Both are necessary for covariation between morphological traits to arise, but they play different roles in the mechanisms that produce covariation. Direct connections between developmental pathways generate regular associations among morphological traits by acting as conduits for variation regardless of its origin. For parallel variation of separate developmental pathways, however, the regularity of the association arises from the source of variation itself, which generates covariation through its simultaneous effects on multiple pathways.

The theoretical framework of Cowley and Atchley (1992) distinguishes the effects of developmental interactions among traits as epistatic effects from the intrinsic pleiotropic effects that genes exert on separate traits simultaneously. Their concept of epistatic effects of a gene on multiple traits approximately corresponds to pleiotropy by direct connection between developmental pathways. Likewise, their notion of intrinsic pleiotropy is more or less equivalent to pleiotropy by parallel effects of genes on separate developmental pathways. Cowley and Atchley make this framework amenable for statistical analysis by assuming that

the effects are additive, that means, that the system is linear. In general, however, developmental processes are nonlinear, and often extremely so, and it cannot be assumed that any rescaling of phenotypic values is able to linearize the effects of all processes simultaneously. In a developmental system of multiple nonlinear and interdependent processes that is not known completely, therefore, it is unlikely that epigenetic effects and intrinsic pleiotropy among traits as proposed by Cowley and Atchley (1992) can be separated by statistical means. This theoretically elegant approach will therefore not be practical for empirical studies of developmental integration.

A morphometric approach to delimit developmental modules

Developmental modules can be recognized as those spatial domains of organisms within which there is strong integration through direct developmental interactions, and which are relatively independent of other such domains. Therefore, to identify developmental modules from morphological data, covariation due to direct connection of developmental pathways is informative, but not covariation from parallel variation of separate pathways (see also Klingenberg 2002a). To isolate covariation due to direct connection of developmental pathways, it is desirable to control rigorously for environmental and genetic variation, because that would eliminate the variation leading to parallel variation of separate pathways.

A straightforward biometric protocol that contains an inherent control for genetic and environmental factors is to analyze patterns of covariation in fluctuating asymmetry. Fluctuating asymmetry refers to small random differences between corresponding parts on the left and right body sides of each individual (e.g., Palmer and Strobeck 1986; Palmer 1994; Møller and Swaddle 1997). The left and right body sides share the same genome and in most organisms also very nearly the same environment. Because they are "held constant" between the body sides of each individual, genotype and environment cannot produce left-right asymmetries, nor can genotype × environment interactions. This argument assumes that phenomena like somatic mutation and somatic recombination are rare, and it may not apply to sessile organisms located in an environmental gradient, but it should hold at least for most mobile animals (Klingenberg 2002b). Therefore, the structures arising on either body side are

replicates of each other that develop separately under nearly identical conditions, and, in a completely deterministic system, would be identical mirror images of each other. Development is not strictly deterministic, however, and there are small random perturbations during development differences between corresponding morphological structures on the left and right body sides. Random variation from many developmental processes can generate such fluctuating asymmetry, because the dynamics of most cellular processes are inherently stochastic (McAdams and Arkin 1999; Klingenberg 2002b), but it must originate within the developmental system itself.

Covariation in fluctuating asymmetry between traits can only arise through direct connections between their developmental pathways. Because the perturbations responsible for the asymmetry originate within the pathways themselves, they can only generate covariation of asymmetry if the perturbations themselves are transmitted between pathways through direct connection. Completely separate pathways also can show fluctuating asymmetry, but the asymmetries are uncorrelated because perturbation cannot be transmitted among pathways. Therefore, the analysis of covariation in fluctuating asymmetry is a way to isolate the contribution of direct connections between developmental pathways to the integration among traits. Comparing the patterns of covariance in asymmetry to the patterns of covariance among individuals, which also includes a contribution from parallel variation of separate pathways, will then make it possible to assess the importance of both ways of generating morphological integration (Klingenberg and Zaklan 2000; Klingenberg et al. 2001; Klingenberg 2002a).

These analyses focus on the covariation of fluctuating asymmetries, that is, the joint variation of asymmetry in multiple variables around the average asymmetry. Therefore, such analyses automatically correct also for directional asymmetry, the systematic difference between the averages of traits on the left and right sides, as it is commonly found in subtle form even in structures that superficially appear symmetric (Klingenberg et al. 1998; Klingenberg 2002b).

These analyses of covariation of fluctuating asymmetry for studying the developmental basis of morphological integration differ in important ways from other

analyses of fluctuating asymmetry in multiple traits (e.g., Lens and van Dongen 1999; Leung et al. 2000). Those analyses examine whether individuals differ consistently in the amount of asymmetry in different traits, reflecting variation in the organism-wide capacity to buffer against developmental perturbation. Therefore, those analyses consider traits that are developmentally independent of one another, so that different traits can be used as independent sources of information. Those studies also use the absolute values of asymmetries (unsigned asymmetry), because it is the magnitude and not the direction of asymmetry that is of interest. In contrast, to identify developmental modules, it is essential that signed asymmetries are analyzed (e.g., raw right – left differences for each variable), because the directions of asymmetries are of critical importance for analyzing the covariation among traits (Klingenberg 2002a).

Covariation of signed asymmetries for linear distance measures has long been documented (e.g., Jolicoeur 1963; Leamy 1984, 1993; Hallgrímsson 1998), but these studies did not specifically examine the developmental relationships among traits (but for a partial attempt, see Sakai and Shimamoto 1965). In recent years, the methods of geometric morphometrics have been adapted to study left-right asymmetry (e.g., Klingenberg and McIntyre 1998; Auffray et al. 1999; Klingenberg et al. 2002). This approach offers a particular potential for delimiting developmental modules, because it explicitly takes into account the geometry of patterns of variation, and therefore facilitates their interpretation in the anatomical context of the structure under study. These geometric methods have been applied for the comparison of covariance patterns between fluctuating asymmetry and individual variation (Klingenberg and McIntyre 1998; Debat et al. 2000; Klingenberg et al. 2002) and specifically for delimiting developmental modules (Klingenberg and Zaklan 2000; Klingenberg et al. 2001).

Only a few studies have used this approach so far, which have confirmed the feasibility of the method and have produced some first results (for a more detailed review, see Klingenberg 2002a). A morphometric study of *Drosophila* wings (Klingenberg and Zaklan 2000) examined the question whether the entire wing is a single module or whether the anterior and posterior compartments, which are separate cell lineages from the inception

of the wing imaginal discs (Held 2002, pp. 87–91), are distinct modules. The study found that fluctuating asymmetry is almost completely integrated throughout the wing, because the component of variation shared between the two compartments accounted for nearly all the variation across the entire wing. These results indicated that the entire wing is a single coherent module, and that the anterior and posterior wing compartments are not separate modules (Klingenberg and Zaklan 2000). This agrees well with results from developmental biology indicating that the boundary between the anterior and posterior compartments is the source of signals that are critical for patterning in both compartments (Held 2002, ch. 6). The boundary is therefore not an inert delimiter between compartments, but is itself an active center of integration throughout the wing.

A further study showed that the fore- and hindwings of bees are each an integrated module and clearly separated from one another (Klingenberg et al. 2001). Accordingly, in flies and bees, each entire wing constitutes a module, which presumably relates to the fact that each wing is derived from a separate imaginal disc and that the signaling interactions or other processes taking place within each disc provide strong integration.

Moreover, the studies of fly and bee wings also have found good agreement between the covariance patterns for fluctuating asymmetry and variation among individuals (Klingenberg and McIntyre 1998; Klingenberg and Zaklan 2000; Klingenberg et al. 2001). This agreement suggests that the same processes may be responsible for covariation of asymmetry as well as of variation among individuals, and in particular, that direct connections among developmental pathways also may have an important or even dominant role in shaping genetic and environmental components of covariance in insect wings.

In contrast, a study of mouse skulls found considerable discrepancies between the covariance patterns for fluctuating asymmetry and individual variation, and suggested that different processes were responsible for each (Debat et al. 2000). A comparable result with no similarity between covariance patterns for fluctuating asymmetry and variation among individuals was also obtained in a small study of pharyngeal jaws in a species of cichlid fish (Klingenberg et al. 2002). In that case, the dominant pattern for inter-individual variation may correspond to phenotypic plasticity associated with the trophic polymorphism of this

fish, and thus represents an outside factor fundamentally different from the developmental processes controlling integration for fluctuating asymmetry. Clearly, the few studies that are available so far are not sufficient for a generalization of the results. Nevertheless, some interesting patterns have emerged, which indicate that further research, particularly on more complex structures such as whole skulls, will be worthwhile.

Modules, integration, and the evolution of pleiotropy

Integration and modularity have often been discussed in an evolutionary context, frequently with the connotation that they are both adaptive themselves. Integration evolves to ensure that different parts and organ systems are coordinated into a whole functioning organism. Modularity, however, allows for evolution in some body parts without effects on others, and thereby provides an escape from the universal trade-offs between organismal functions as they would exist in a completely integrated organism. Therefore, an important question is how patterns of integration themselves evolve. Some authors have argued that genetic covariance matrices evolve to reflect the multivariate selection regime and the functional relationships of the morphological traits (Cheverud 1984; Cheverud 1996; Wagner 1996; Wagner and Altenberg 1996). So far there are no empirical studies, however, that clearly document the adaptive evolution of patterns of variation and rule out nonadaptive alternatives. In these considerations, a crucial issue is the evolution of the patterns of pleiotropy for the genes involved.

This chapter offers a new perspective on this issue, which emphasizes development by distinguishing the different ways in which pleiotropy can originate (Hadorn 1945; Pyeritz 1989; Hodgkin 1998; Wilkins 2002, p. 117–118). A gene can have simultaneous effects on multiple traits either by direct connection between developmental pathways or by their parallel effects on multiple separate pathways (Fig. 2). Clearly, both mechanisms can cause genes to have similar effects on the phenotype, since both can produce pleiotropy. A quite different question, however, is whether these two distinct developmental sources of pleiotropy also have the same potential for evolutionary change (see also Cowley and Atchley 1992).

With direct connection between developmental pathways, any gene that affects the pathway upstream of the connection ("variable step" in figs. 2A, B) will have a pleiotropic effect on all the descendant traits because allelic effects are transmitted between pathways. Moreover, provided that allelic variation leads to differences in the activities of relevant gene products that can be transduced through the pathway, multiple "upstream" loci will have congruent patterns of pleiotropy due to the same connection of pathways. Evolution of the patterns of pleiotropy must therefore occur by changing the linkage among pathways itself, for example, by changes in signaling or the mechanism of partitioning a developmental field. These changes may have profound effects on the resulting morphological structure and its function. In other words, it is likely that these changes in signaling mechanisms will often be under stabilizing selection and that patterns of pleiotropy through direct connection of developmental pathways will be fairly conservative. Because direct connections between developmental pathways occur primarily within modules and only to a lesser degree between modules, the evolutionary conservatism of the resulting patterns of pleiotropy will contribute to the evolutionary inertia of the modular organization itself. If a change in the connection of developmental pathways is selectively advantageous, it can be a source of morphological innovation. Such an evolutionary transition to a novel interaction between pathways could then lead to a complete reorganization of the spatial pattern of the module, and therefore to a concerted change in the patterns of pleiotropy for all the genes upstream of the link between pathways.

In contrast, pleiotropy by parallel effects of a gene on multiple developmental pathways relies entirely on the activity of that gene alone. Because transcriptional control of genes is itself generally modular, the expression of the gene in each separate developmental context is normally controlled by one or a few separate enhancer elements (Davidson 2001). In order to exert a joint effect on two different pathways, allelic differences must lie either in a *cis*-regulatory element that is activated in both pathways, or they must affect the transcript itself (either by a difference in the protein-coding sequence or in untranslated regions affecting posttranscriptional processing and the control of translation). Therefore, pleiotropy by this mechanism requires a particular kind of allelic variation to exist in a population.

There can be a great diversity of patterns of pleiotropy, however, because every allele of a gene can have a distinct combination of effects on different developmental pathways. These patterns of pleiotropy can be modified by mutations that affect the expression of the gene; for instance, any regulatory changes that lead to reduced expression of the gene in a subset of pathways can reduce the pleiotropic effects of allelic variation at that locus. Because the relevant changes in *cis*-regulatory regions can occur rapidly (Stone and Wray 2001), pleiotropy by parallel effects of a gene on multiple pathways is likely to evolve readily under natural selection.

Evolution of genetic covariances

Just as the developmental origin of morphological covariation makes a difference for the evolution of the pleiotropic effects of single loci, it can also affect changes of genetic variances and covariances in natural populations, which are due to the aggregate effects of all segregating loci. The evolution of genetic variances and covariances among traits is an important issue in evolutionary quantitative genetics, because long-term predictions of response to selection or of random drift depend on the genetic covariance matrix (reviewed by Roff 1997, and this volume). Genetic covariances can be due to pleiotropic effects of individual loci, but they also can arise from genetic linkage among loci that affect different traits (e.g., Lynch and Walsh 1998). As outlined above, pleiotropic effects can originate through direct connection or parallel variation of developmental pathways. The origin of covariance by genetic linkage is a special case of parallel variation, in which different developmental pathways are affected by different loci whose effects are associated statistically by the genetic linkage.

If direct developmental linkages between developmental pathways contribute most of the covariation between traits, shifts in allele frequencies will have relatively small effects on the patterns of covariance. Because the connections of pathways act as a common conduit for the effects of multiple “upstream” genes, the patterns of genetic covariance will be similar regardless of the specific allelic differences and allele frequencies in a population. Direct links among developmental pathways will therefore contribute to the constancy of

covariances among traits. Because of the strong direct interactions among the parts of a developmental module, this reasoning suggests that patterns of genetic covariances among traits within a module should be relatively stable, even over evolutionary time scales.

In contrast, if covariation among traits arises primarily by parallel variation of separate developmental pathways, the patterns of covariation will be more labile. Because every allele can have different combinations of pleiotropic effects, genetic covariances will depend strongly on allele frequencies in the population. Because genetic linkage is also subject to change in natural populations, the genetic covariances produced by it will also be evolutionarily fluid. Patterns of genetic covariances due to parallel variation of separate developmental pathways are therefore be likely to undergo substantial evolutionary transformations by selection and drift.

Conclusions

This chapter has reviewed the developmental origin of morphological integration and examined its implications for evolution. Morphological integration reflects the fact that organisms and their development are organized into modules. Most adult body parts arise from distinct morphogenetic fields within which spatial pattern is established by direct developmental interactions, which also integrate the components of the module into a coherent unit. The resulting morphological integration is manifest in genetic as well as non-genetic components of variation. The spatial extent of modules can be delimited by analyzing the patterns of covariation for fluctuating asymmetry, which indicate the domains within which there is integration by direct developmental interactions. Although it may seem paradoxical at first, it is possible to use this approach that is based on variation of non-genetic origin to study the developmental basis of pleiotropy and genetic integration.

Developmental integration by direct interactions within modules is one of the prime factors determining patterns of pleiotropy. It is likely that these patterns of pleiotropy are evolutionarily conservative, because to change them would require fundamental alterations of the developmental processes involved. In contrast, whole-organism integration across modules, by parallel variation of separate developmental pathways, relies on a different

mechanism and requires an extrinsic source of variation. It is likely that pleiotropy due to this process can evolve easily by regulatory changes in the genes responsible. Similarly, the developmental origins of genetic covariances at the population level are important determinant for their evolution. Again, it is likely that patterns of genetic covariance that are due to direct developmental interactions within modules are more robust evolutionarily than covariances due to parallel variation of separate developmental pathways.

Clearly, the ideas and hypotheses presented here need to be developed further and tested empirically, but they have the potential to provide a new perspective on the role of development for genetic and phenotypic integration among traits. A developmental perspective offers a framework for obtaining a unified understanding of morphological variation, from molecular mechanisms to phenotypic manifestation. Inclusion of information on gene regulation, signaling, and the molecular basis of growth and differentiation has much to offer to evolutionary quantitative genetics.

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Figures and legends

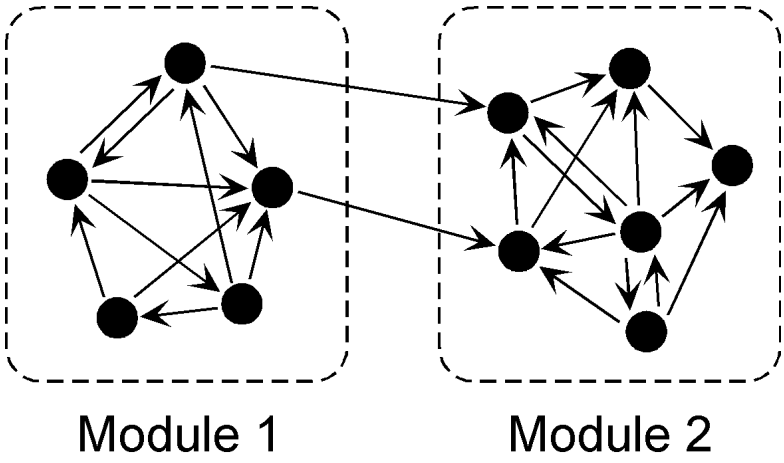

Figure 1. Definition of modules by developmental interactions. Component parts within modules are interconnected by many interactions, whereas there are fewer interactions between modules.

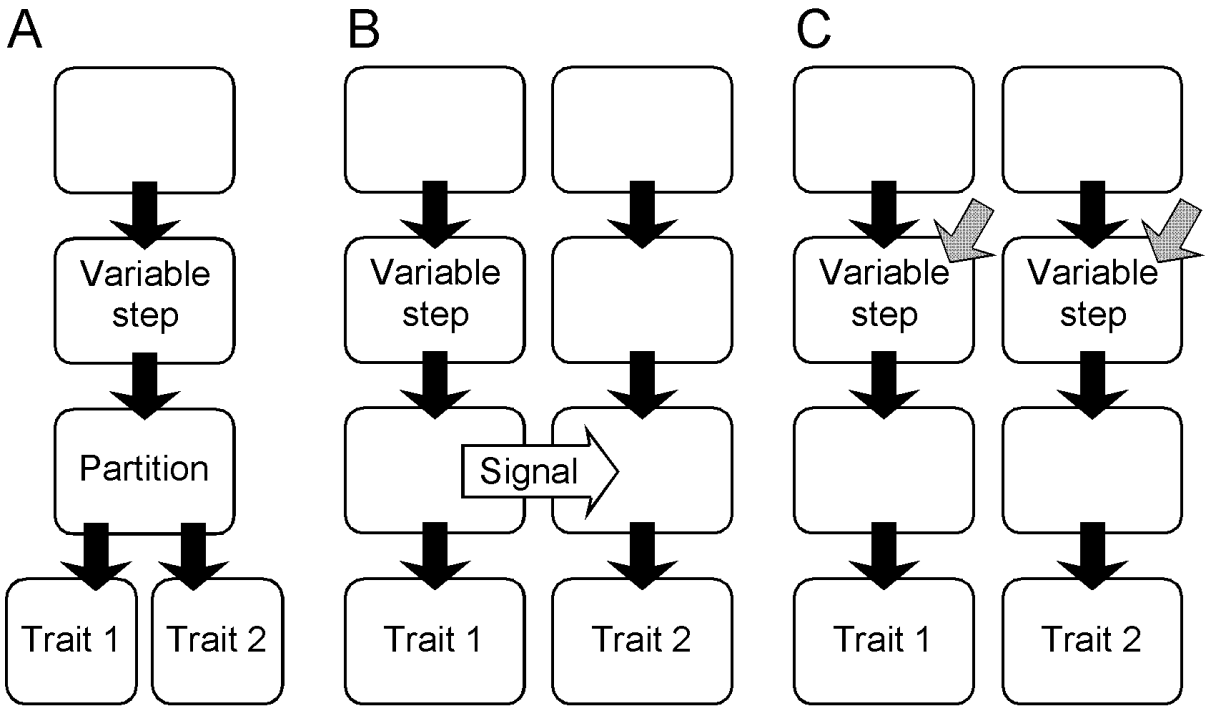

Figure 2. Origins of covariation between morphological traits (modified after Klingenberg 2002a). (A) Direct connection between pathways of two traits due to partitioning of a common developmental precursor. The variation existing in the pathway before or at the partition is transmitted and can manifest itself as covariation between the traits. (B) Direct connection by signaling between pathways. Variation is transmitted from the pathway containing the source of the signal, and therefore can jointly affect the traits that arise from both pathways. (C) Parallel variation of two separate developmental pathways. Because there is no transmission of variation between pathways, covariation relies entirely on the simultaneous effects on both developmental pathways by an extrinsic source of variation (gray arrows).