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Modularity in Development and Why It Matters to Evo-Devo[1]

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SYNOPSIS. The concept of modularity is fundamental to research in both evolutionary and developmental biology, though workers in each field use the idea in different ways. Although readily and intuitively recognized, modularity is difficult to define precisely. Most definitions of modularity are operational and implicit, particularly in developmental biology. Examination of several proposed definitions points to some general characteristics of developmental modules, for example their internal integration, and suggests the importance of devising a definition applicable at different levels of the biological hierarchy. Modules, like homologs, must be defined with respect to a specified level of the hierarchy, and a general definition should support both analyses of the evolving causal relationships between levels, and studies of the interconnections between modules of the same type. The designation of a developmental structure, process, or function as a ''module'' is a testable hypothesis; this hypothesis is confirmed in the case of the dorsal marginal zone of the amphibian gastrula, which acts as a morphogenetic module. Discussions of developmental modularity can provide a meeting place for developmental and evolutionary biologists by helping us articulate key questions at the intersection of the two fields, and design experiments to begin answering them.

INTRODUCTION

Both evolutionary and developmental biologists have used the concept of modularity to describe and explain patterns of metazoan organization, from body segmentation to the structure of signal transduction pathways. Definitions of ''modularity'' vary widely; nevertheless, implicit assumptions of modular organization are central both to studies of developmental mechanisms, and to evolutionary analyses of morphology, physiology, and phylogeny.

In developmental biology, an implicit hypothesis of modularity is embodied in the working assumption that one can experimentally divide a developing organism into functional or organizational subunits. The clearest examples of developmental subunits or modules are embryonic structures that show clear morphological individualization (*e.g.*, somites), or correspond directly to distinct elements of adult morphology (*e.g.*, limb buds). Explorations of developmental mechanisms have led to the recognition of additional modules, such as the limb morphogenetic field, that are defined on the basis of their developmental potential.

In evolutionary biology, a hypothesis of modularity serves as the basis for identifying and studying individual elements of an organism's genotype or phenotype. Most of the analytical tools for describing the evolution of organisms rest (explicitly or otherwise) on the premise that one can delineate distinct characters. Phylogenetic analyses depend on determining the homology of the chosen characters; however, this assessment in turn depends on the prior recognition of those features as evolutionarily discrete modules.

Explicit discussions of modularity have often focused primarily on its evolutionary significance, or on modularity-based methods in evolutionary biology. Recently, developmental biologists have been paying increasing attention to the concept, for two reasons. First, we now have enough information about specific developmental genes and pathways to start thinking about the overall principles and patterns that describe their organization (*e.g.*, von Dassow *et al.*,

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2000; Arnone and Davidson, 1997; Kirchamer *et al.*, 1996). Second, the renewed emphasis on evolutionary developmental biology has directed attention to the structure of development as a whole, and how that structure may be transformed through phylogeny as well as during individual ontogenies. For example, studies of limb development across a wide range of animal species have explored the relationship between evolutionary patterns and developmental processes in a system that has long served as a model for biologists in both fields (Shubin *et al.*, 1997; Raff, 1996; Sordino *et al.*, 1995; Carroll *et al.*, 1995).

DEFINING MODULARITY

Formulating a definition of modularity that is both comprehensive and practical is a non-trivial task. It is surprisingly hard to define something we easily recognize in the biological world, namely its organization into individualized yet interconnected units across a range of physical and functional scales. Part of the difficulty may be precisely that it is often easy to recognize modularity, and to develop practical, working definitions that are never made explicit. For example, evolutionary biologists and morphologists readily identify the tetrapod forelimb as a discrete structure that is homologous across different taxa, despite its structural, functional and adaptive diversity. Developmental biologists recognize the limb bud as an embryonic region with unique intrinsic patterning and developmental integration that can be physically displaced or induced ectopically, yet retains its fundamental structure and identity. Each of these descriptions serves as a working definition of modularity for the limb in the context of a specific research program.

Practical vs. *formal definitions*

Working hypotheses of modularity are operational definitions, devised and refined according to the particular experimental organism, system, or circumstances. They may not be philosophically explicit, but they work. We can draw a useful analogy to the historical understanding of chemical elements.[3] Early definitions of chemical elements were accurate and useful, although they were formulated in the absence of a theory that could explain elemental properties at a mechanistic level. Even without such a theory, the properties themselves were clear. Indeed, understanding the operational characteristics of different elements not only permitted experimental progress in advance of a theory, but contributed to the eventual development of mechanistic explanations for properties that were already reliably established.

The alternative to an operational definition is an explicit, formal, or theoretical description. The ideal way to arrive at a formal definition of developmental modularity would be to derive it from a general theory of development, following Lewontin and Hull's elegant approach to the units of selection problem in evolutionary biology (Lewontin, 1970; Hull, 1980). Starting from Lewontin's description of the mechanism of natural selection (Lewontin, 1970), Hull sought to identify not ''which entities have the characteristics necessary to function in the evolutionary process . . . [but] . . . the precise nature of these general characteristics'' (Hull, 1980, p. 315). He concluded that any entity that functions as an ''individual'' with respect to the three fundamental processes of replication, interaction, and evolution (Lewontin, 1970) can be a unit of selection. Rather than focus on the entities or units undergoing a process (in this case, evolution by natural selection), he considered what characteristics the process itself demands of participants.

Wagner has suggested generating a definition of modularity in a similar way (Wagner, 1996). The difficulty in applying this method to developmental modularity is that we still lack a unifying, mechanistic theory of development comparable to the central Darwinian theory from which Lewontin derived his ''replication, interaction, and evolution.'' Until we can frame such a theory, this approach remains closed, at least for a general definition. We can, however, devise

[3] I am grateful to Günter Wagner for suggesting this comparison.

more local—and still useful—definitions of modularity.

Some definitions of developmental modularity

The most local definitions of modularity are closely tied to a specific context, and based on functional criteria and tests within that context. One example is Arnone and Davidson's definition of *cis*-regulatory modules of gene expression: ''a fragment of *cis*-regulatory DNA that, when linked to a reporter gene and transferred into an appropriate cell, executes a regulatory function that is a subfraction of the regulatory function executed by the complete system'' (Arnone and Davidson, 1997, p. 1852). Regulatory modules of gene expression can be discovered, or putative modules tested, by a well-defined experimental strategy (Arnone and Davidson, 1997; Kirchamer *et al.*, 1996). (Hartwell *et al.* [1999] suggest a similar approach to defining functional modules in cell biology.) Such local definitions of modularity are restricted to a single context, or at most a single level of the biological hierarchy, precisely because they are based on particular functions or mechanisms within that context. They have great power within a level, but limited ability to bridge different levels.

Broader generality is offered by Atchley and Hall's description of ''fundamental developmental units'' (or modules), which they define as ''those basic structural entities or regulatory phenomena necessary to assemble a complex morphological structure'' (Atchley and Hall, 1991, p. 112). These units are derived from observations of specific developmental events, in this case the formation of cartilaginous condensations, that can be linked to particular morphological outcomes (here, the development of the mammalian dentary). The units Atchley and Hall propose for this system include the number of stem cells in each condensation, relative time of condensation initiation, rate of cell division, fraction of the cells that are dividing, and rate of cell death (Atchley and Hall, 1991).

Unlike Arnone and Davidson's definition of a module, Atchley and Hall's does not require that underlying mechanisms be thoroughly characterized. In fact, carefully defining developmental units or modules is a key first step in the pursuit of underlying genetic mechanisms, whether by genetic mapping or by candidate gene approaches (Streelman and Kocher, 2000; Hall and Miyake, 2000; Atchley and Hall, 1991). Atchley and Hall sought to define developmental units in order to facilitate the search for both underlying mechanisms, and larger-scale patterns of morphological evolution—that is, to help bridge different levels of the biological hierarchy. While their definition effectively connects the levels immediately above and below their focal level, that of morphogenetic processes, it is difficult to apply more generally.

Raff (1996) avoids many of the limitations of context-specific definitions by listing a series of characteristics of developmental modules, rather than starting with any particular developmental phenomenon. Modules should have discrete genetic specification, hierarchical organization, interactions with other modules, a particular physical location within a developing organism, and the ability to undergo transformations on both developmental and evolutionary time scales (Raff, 1996). Raff describes modules as not merely physical parts or regions of embryos: rather, they are ''dynamic entities representing localized processes (as in morphogenetic fields) rather than simply incipient structures . . . (. . . such as organ rudiments)'' (Raff, 1996, p. 326). He then illustrates how these criteria apply to the particular case of the tetrapod limb. By focusing on a developmental phenomenon that has been studied intensively on many different levels, from genetic to paleontological, Raff is able to assess and confirm its modularity according to each of his proposed characteristics. The drawback to this set of criteria is that one needs a great deal of information up front: they work beautifully in the case of the limb, but would be much more difficult to apply to a less well-studied example. In the latter case, however, Raff's criteria serve to outline a research agenda that would yield a deeper understanding of the system, and eventually allow assessment of its modularity.

What general principles or components

of a definition of modularity can we extract from these three examples? First, a useful definition of modularity should work across a broad range of cases, not just apply retrospectively to those we already know well; ideally, it will also help guide research on more obscure systems. Next, it should include the following points: (1) a module is a biological entity (a structure, a process, or a pathway) characterized by more internal than external integration. (Von Dassow and Munro [1999] discuss such ''connectivity criteria'' at length.) (2) Modules are biological individuals (Hull, 1980; Roth, 1991) that can be delineated from their surroundings or context, and whose behavior or function reflects the integration of their parts, not simply the arithmetical sum. For example, a vertebrate neural plate or keel can differentiate into a neural tube of a particular shape that exhibits characteristic spatial and temporal patterns of gene expression. Its constituent cells, dissociated, can't do those things. What distinguishes modules (as defined here) within the broader category of ''individuals'' is that modules have external connectivity, in addition to their internal integration: (3) a module can be delineated from other entities with which it interacts in some way. A cell or a morphogenetic field within an embryo is a module; the embryo itself, though an individual, is not. Finally, the definition of modularity should work across multiple levels of the biological hierarchy. This last criterion applies to the definition, not to the modules themselves; the object is to be able to compare patterns of modularity at different levels, and examine their relationships (see below).

IDENTIFYING A MORPHOGENETIC MODULE

There are two general ways to test a hypothesis of developmental modularity. Experimental assays are the ideal way to examine modular processes and functions, while modular patterns and structures are more readily identified using a comparative approach. Most developmental studies center on experimental analyses: dissociation of what are, effectively, developmental modules. Experimental tests of developmental modularity can take many forms, from physical manipulation of embryos to molecular genetic approaches. I will focus here on experimental analyses of a morphogenetic process: the behavior of the amphibian dorsal marginal zone during gastrulation.

Morphogenesis is fundamentally a physical phenomenon: changes in the shape of embryonic tissues depend on the generation of specific forces at the correct times and locations, on the response of cells and tissues to applied forces, and on the integration of mechanical processes within and between parts of the embryo (Koehl, 1990). Therefore, analyzing morphogenesis requires manipulation and isolation of different tissues, to generate and test hypotheses about both force-producing mechanisms, and the factors that determine their results. These factors range from the physical characteristics of the tissue to which the force is applied, to its mechanical context within the embryo (Moore *et al.*, 1995; Bolker, 1993; Adams *et al.*, 1990; Koehl, 1990).

The best-characterized morphogenetic module is the dorsal marginal zone (DMZ) of the *Xenopus* gastrula (Keller, 1986). Lengthening and narrowing (convergent extension) of this well-defined region of the embryo is the primary ''motor'' that drives extension of the prospective neural plate and axial mesoderm, and involution of the dorsal mesoderm and endoderm that line the roof of the gastrocoel (Keller, 1984; Keller *et al.*, 1985*a*, *b*; Keller and Danilchik, 1988).

Why label the dorsal marginal zone a ''module''? First, it can be physically isolated from the rest of the embryo, and nevertheless undergo its characteristic shape change, showing that the mechanism for convergent extension is intrinsic to the DMZ (Keller and Danilchik, 1988). Isolated explants have also been used to measure directly the force generated by the extending tissue (Moore *et al.*, 1995). Second, the force-generating function is uniquely localized to this tissue: removing the dorsal marginal zone from a *Xenopus* embryo prevents gastrulation, though removing the adjacent blastocoel roof does not (Keller and Jansa, 1992). Third, the directed protrusive activity of cells within the DMZ that gen-

erates its overall shape change is precisely patterned, and depends on cell–cell communication within the tissue (Domingo and Keller, 1995; Keller *et al.*, 1992*a, b, c*). Fourth, the homologous region can be identified not only in other amphibian embryos, but also in the distantly related white sturgeon, where analogous experiments reveal that the DMZ carries out a similar morphogenetic function (Bolker, 1993). Moreover, altering the mechanical context of DMZ extension in sturgeon (by removing the blastocoel roof, so that the DMZ is at the equator of the embryo rather than near the vegetal pole when convergence begins) abolishes its morphogenetic function, without affecting its intrinsic behavior (Bolker, 1993).

The dorsal marginal zone fulfills the necessary criteria for a module. It has significant internal integration, as evidenced by its ability to converge and extend in isolation from the rest of the embryo, and by the necessity for cell–cell communication within the tissue. Although the DMZ can carry out its characteristic behavior by itself, the net morphogenetic function of that behavior (driving extension and involution) depends on its context within the intact embryo, particularly in the sturgeon. Finally, identifying the DMZ as a morphogenetic module in *Xenopus* serves as a basis for further studies seeking related mechanisms and modules at the cellular and genetic levels (*e.g.*, Kim *et al.*, 1998), as well as for comparative studies in other vertebrates (Shook *et al.*, 2000; Minsuk and Keller, 1996; Purcell and Keller, 1993; Bolker, 1993; Lundmark *et al.*, 1984).

Modules and Levels

Modules can exist at different levels of the biological hierarchy; they must therefore be defined with respect to a specified level, and to the processes that occur at that level (Hull, 1980; Lewontin, 1970; Striedter and Northcutt, 1991 make the identical argument for homologs). We can recognize modularity across a range of scales or levels, from nucleotide sequences to behavior, but there is not an isomorphic mapping from one level to the next, nor can higher levels necessarily be reduced to lower ones (Striedter and Northcutt, 1991). For example, there is no ''gene for'' any single morphogenetic process (though single gene lesions can cause remarkably specific disruptions of morphogenesis; *e.g.*, Solnica-Krezel *et al.*, 1996).

Most importantly, the causal relationships between entities at different levels of the biological hierarchy evolve. Because modules exist at many different levels, the mapping between different kinds of modules (for example genetic and morphological) can evolve, just as structural homologs may be underlain by different morphogenetic processes, or behavioral homologs by different structures (Striedter and Northcutt, 1991; de Beer, 1971). Such shifts in causal relationships between levels can have evolutionary significance in their own right or, in other cases, reveal constraints that can influence evolutionary events and trajectories at other levels (Wagner and Gauthier, 1999; Shubin *et al.*, 1995).

Modularity in Evo-Devo

One historical difference between evolutionary and developmental biologists has been that, for the most part, they work at different levels of biological organization. They thus tend to be aware of different types of modules. Moreover, researchers in evolutionary and in developmental biology may apply the concept of modularity in complementary ways. Evolutionary biologists describe modules as subunits or components of a larger system (*e.g.*, specific morphological elements of a whole organism). In contrast, developmentalists often use the term to refer to a set of lower-level components (such as individual genes) that act in a unified way, or together perform a given function: a module in this sense is a collective, rather than a subunit (von Dassow *et al.*, 2000; Hartwell *et al.*, 1999).

The recognition of biological modularity by both developmental and evolutionary biologists allows the concept to serve as a meeting place for the two disciplines. This meeting is facilitated by convergence of evolutionary and developmental discussions of modules on similar levels of biological organization. Developmentalists are building up from smaller units, and starting

to combine detailed knowledge of individual genetic and cellular components to describe modules of process, morphogenetic function, and so on. These synthetic modules are starting to approach the level of biological organization to which evolutionists traditionally break things down, for example morphological elements or patterns of growth. Tracking the correspondence, or lack thereof, between a developmentalist's ''module'' and an evolutionist's should be a very interesting exercise (von Dassow and Munro, 1999).

For developmental biologists, explicitly recognizing modules is a critical step toward framing and testing hypotheses about their evolutionary origins and significance. Such focused developmental analyses can elucidate for evolutionists the mechanistic basis for modules they already recognize as evolving phenotypic elements. For evo-devo, studying the developmental assembly and integration of modules is central to understanding how they and their interconnections may originate, break down, and change through evolutionary time.

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