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Modularity in Animal Development and Evolution: Elements of a Conceptual Framework for EvoDevo

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ABSTRACT For at least a century biologists have been talking, mostly in a black-box sense, about developmental mechanisms. Only recently have biologists succeeded broadly in fishing out the contents of these black boxes. Unfortunately the view from inside the black box is almost as obscure as that from without, and developmental biologists increasingly confront the need to synthesize known facts about developmental phenomena into mechanistic descriptions of complex systems. To evolutionary biologists, the emerging understanding of developmental mechanisms is an opportunity to understand the origins of variation not just in the selective milieu but also in the variability of the developmental process, the substrate for morphological change. Ultimately, evolutionary developmental biology (EvoDevo) expects to articulate how the diversity of organic form results from adaptive variation in development. This ambition demands a shift in the way biologists describe causality, and the central problem of EvoDevo is to understand how the architecture of development confers evolvability. We argue in this essay that it makes little sense to think of this question in terms of individual gene function or isolated morphometrics, but rather in terms of higher-order modules such as gene networks and homologous characters. We outline the conceptual challenges raised by this shift in perspective, then present a selection of case studies we believe to be paradigmatic for how biologists think about modularity in development and evolution. *J. Exp. Zool. (Mol. Dev. Evol.) 285:307–325, 1999.*

Biologists have long recognized that the evolvability of development is the key to morphological evolution. However, only recently has molecular genetics produced substantial characterizations of developmental regulatory processes and thus made it possible to analyze them comparatively. This has fueled resurgent interest in the evolution of development, but evolutionary developmental biology (EvoDevo) lacks a clear conceptual framework. Blithe attempts to apply classical comparative concepts to development lead more often than not to confusing or contradictory results. Furthermore the idea that one should focus on developmental mechanisms as units of evolutionary change begs the question, what constitutes a developmental mechanism?[1]

Classical accounts of animal form concentrate on identifying morphological characters and on tracing their phylogenetic modification. The dichotomous notions of homology and analogy are implicitly predicated on a modular view of animal design; their ease of use is proportional to the ease with which one can identify the modules. Embryogenesis, too, consists of logically separable processes—gastrulation, establishment of body axes or morphogenesis of individual organs or appendages—and we take for granted that logical separability reflects underlying modularity: the experimental study of development assumes that one may meaningfully isolate (physically or conceptually) and study individual processes independent from one another. Functional decomposability is thus a necessary presumption to considering developmental mechanisms either as units of explanation within de-

[1]This essay arose from a workshop on modularity in development and evolution organized by the authors and Dr. Evelyn Fox Keller, held in September 1997 at the Friday Harbor Laboratories. In addition to the authors, the participants were Drs. Jessica Bolker, Richard Burian, Marie-Anne Félix, Scott Gilbert, Jason Hodin, Evelyn Fox Keller, Eli Meir, Jay Mittenthal, Lisa Nagy, Garrett Odell, Jarmila Kukalova-Peck, Louise Roth, Günter Wagner, and William Wimsatt. To the workshop proceedings and participants we owe much of the development of our thinking on the topics discussed in this essay and several of the examples discussed, and we are forever grateful to the participants for sharing their thoughts and collegiality both during the workshop and after. We express our appreciation to the Friday Harbor Laboratories, its staff, and director for a wonderful environment for a meeting. We are also especially grateful to Dr. Evelyn Fox Keller for encouraging us in these directions, for the idea of a workshop on modularity, and for providing the bulk of the funding for that workshop out of her own pocket.

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velopment or as units of evolutionary change. Despite the pervasiveness of this implicit assumption, and despite its importance as a pre-requisite to mechanistic explanations, there exist only rudiments of a developmental modularity concept.

Those rudiments are the many intuitive notions about modularity that have attracted so much attention recently (Raff, '96, esp. chapter 10), including morphogenetic fields (Gilbert et al., '96), gene networks (Bonner, '88; Arnone and Davidson, '97), and the several notions of homologues (Hall, '92). Existing ideas about modularity are based on good biological intuitions about the nature of both the developmental and the evolutionary process, and therefore the challenge is to develop generally useful criteria that handle diverse phenomena within a common explanatory framework. In this essay, we discuss why this is not a trivial problem. Our existing concepts in developmental and evolutionary biology shape the ways in which we analyze both development and form, so we start by tracing historically where present ideas about mechanism are coming from and where they converge. Next, we consider two problems with modularity as a conceptual tool, both of which biologists might be stuck with. The way we conceptualize module and mechanism depends both on the reference process (on whether we are interested in morphogenesis or epigenesis, or in the evolutionary process itself) and on whether we are thinking analytically or synthetically. The first arises because we are talking about disjunct types of process in which biological entities participate differently; the second selects our operational criteria. The practical consequence of both is merely that biologists must be careful not to assume any particular correspondence between units that emerge from each perspective. The map between them is itself an architectural problem that merits attention. In the third and fourth sections we consider how modules of animal form and function (morphological characters) relate to modules of developmental process. Whatever the nature of that relationship in either general or specific terms, individual modules must possess a history through phylogeny of functional adaptation. Biologists have become quite comfortable with the idea that morphological characters are units of phenotypic adaptation. If we say the same is true of developmental mechanisms, then we encounter a problem of correspondence. Thus, we need to understand the interface between developmental and evolutionary mechanics, and shape our ideas about character identity to match.

THE EMERGENCE OF MECHANISM IN DEVELOPMENTAL AND EVOLUTIONARY BIOLOGY

A century ago Wilhelm Roux introduced the notion of developmental mechanics (Roux, 1894), a mechanistic analysis of development by experiment. Embryology before Roux's time was largely descriptive and comparative. By focusing on analysis and experiment, Roux and less polemically inclined colleagues effected a profound shift in biological thought, a conceptual thread to which most modern studies of development can be traced (Maienschein, '91). To this lineage we owe such concepts as regulative development, induction, morphogenetic fields, and more. However, Judson ('79) recounts in "The Eighth Day of Creation" in the chapter "Morgan's Deviation" how, shortly after Roux's declaration, T.H. Morgan abandoned embryology for fruit fly genetics, opining that no one could make any progress in developmental mechanics until biology had made sense of heredity. Indeed, despite the conceptual success of experimental embryology, it hit a very rough patch until it could reconcile its differences with genetics.

Experimental embryology ultimately stumbled on its own rocks, the morphogenetic field and the organizer (see Gilbert et al., '96 for review). Needham and others, inspired on the one hand by the success of Spemann, Harrison, and other embryologists in defining specific, causally relevant events in embryos and on the other by the emergence of metabolic and enzymatic biochemistry, sought to develop "chemical embryology" as an experimental study that would reveal mechanisms underlying the various tissue-level phenomena defined by earlier embryological studies. Needham's three-volume *Chemical Embryology* ('31) testifies to both the theoretical richness and the empirical drought that this movement experienced. The organizer, in particular, led straight to confusion, as a frenzy set in to find the substance responsible for its remarkable properties. No such agent was found, or rather too many such agents were found, and embryologists ultimately set aside chemical embryology (see Nieuwkoop et al., '85 for review).

In hindsight we might say today that the chemical embryologists failed only because they sought answers in the wrong place; urea metabolism and other favorites of Needham and his colleagues don't seem like developmental mechanisms as we think of them today. Things might have gone differently for chemical embryologists had they

known enough about genes and their products. Actually, embryologists of that era, as a group, dismissed genetics as irrelevant because no one could explain how genes continually present from egg through adult could have anything to do with epigenesis (Gilbert et al., '96). Jacob and Monod ('61) provided the first widely noticed answer with the demonstration of regulated gene expression in bacterial cells. The *lac* operon laid the groundwork for the emergence of developmental genetics as the modern version of chemical embryology by showing how inherited genes could directly influence physiology in an epigenetic manner.[2]

But is it really just a matter of choosing, say, transcriptional control over urea metabolism? The last decade has seen a replay of the "organizer substance" frenzy with mRNAs and proteins in place of alcoholic tissue extracts, and the prospects seem only slightly improved. We now know of a variety of macromolecules that do interesting things to frog embryos, and this time around there is good evidence that some of them are actually involved in the developmental process (see Lemaire and Kodjabachian, '96; Harland and Gerhart, '97 for reviews). However, despite the emerging body of literature on secreted signals and localized gene expression, there is still no explanation for certain fundamental properties of the organizer and the primary embryonic field. For example, Cooke ('81) demonstrated that when an exogenous organizer is implanted ventrally in a host embryo, despite the famous twinning phenomenon the relative proportions of tissue types remain constant throughout the embryo. How does this phenomenon emerge from what is presently known about neural inducers, organizer-specific gene expression, ventralizing signals, and so on? Is something missing?

Morgan's deviation is finished; Morgan would have returned to developmental mechanics decades ago, as did many *Drosophila* geneticists in the 1960s, 1970s, and since (for example Garcia-Bellido, '98 relates the history of interest in *engrailed*). There's plenty more to learn about heredity, about the organization, transformation and transmission of genetic information, but we certainly know enough about it to explore developmental mechanism in terms of gene function. Unequivocally, we know something meaningful about development from the vast literature of developmental genetics. As a case in point, we know not just that without the *bicoid* gene flies don't form an anterior-posterior axis correctly; we also know *bicoid* is transcribed in nurse cells, the mRNA is deposited in the egg, anchored at the anterior end, then translated early in development; the Bicoid protein forms a gradient in the egg; Bicoid is a transcriptional regulator that binds to target genes (like *hunchback*), which in turn have target genes, and so forth (Driever and Nusslein-Volhard, '88a,b, '89; Macdonald and Struhl, '88; Struhl et al., '89, '92; Hulskamp et al., '90; Pokrywka and Stephenson, '91; reviewed in Lawrence, '92). The point is, developmental genetics reveals more than just a catalog of required parts, and begins to reveal something about what the parts do, which ones interact, and why things go wrong without them.

What's missing is a way to get from this kind of knowledge to a formal understanding of developmental phenomena. Mechanism, per se, is an explanatory mode in which we describe what are the parts, how they behave intrinsically, and how those intrinsic behaviors of parts are coupled to each other to produce the behavior of the whole. This common sense definition of mechanism implies an inherently hierarchical decomposition; having identified a part with its own intrinsic behavior, that part may in turn be treated as a whole to be explained. Historically, developmental biologists haven't been able to take this approach, primarily because of insufficient knowledge of the parts; you can't explain how the whole skeleton works if you've only got a few bones. Instead for explanation we rely primarily on a sort of local causation. The operational approach of developmental genetics, for instance, takes for granted the organism as a working whole, with no general assumptions about the nature of developmental mechanisms beyond the empirical fact that some fraction of mutations have discrete, visible effects. Developmental genetics begins with an induced anomaly (a mutation) and a (hopefully discrete) consequence, then proceeds to decipher a perturbation-to-consequence chain. As above with

[2]Whether or not their paper was in fact a historic stimulant to developmental genetics is beside the point; before Jacob and Monod, the relation between inherited information and dynamic regulated processes in a living animal—epigenesis—was obscure. Thus the elucidation of the *lac* operon, and later studies of gene expression in prokaryotes and their viruses, should perhaps be thought of as the greatest theoretical advance of developmental mechanics in the century since Roux. Almost all other broad-brush concepts in use today by developmental biologists date back to the age of Roux, August Weissman, and Hans Driesch. One notable exception is the morphogenetic field, which was developed by Needham, Hans Spemann, and others. Another is Paul Weiss's notion of "molecular ecology" which surfaces in a number of his essays; Weiss's idea of how to think of gene regulation never seems to have made it into common parlance, but in this essay we make the argument that its time has come. The reader interested in the conceptual lineage of developmental biology is encouraged to turn to an excellent volume compiled by Gilbert ('91).

bicoid, one begins with a mutation and a phenotype at the ends of the chain and fills in the links of cause and effect until it may be said that mutations in *bicoid* lead to some phenotype *because* Bicoid normally binds to the promoter of *hunchback*, causing *hunchback* transcription, etc. The perturbation-to-consequence chain between mutant gene and abnormal phene is then taken as an explanation for the role of part in process. We owe to this habit the prevalence of metaphors like "genetic program" and "developmental pathway"; implicit in this discussion is a reminder that we oughtn't take such metaphors too seriously as literally descriptive of mechanistic architecture. Rather, they are summarily descriptive of a chain of causation that we have been able to tease out analytically from an as-yet-undescribed architecture.

While this kind of explanation allows one some description of what parts (genes and their products) do to each other, it doesn't articulate any sense of the mapping from genotype to phenotype, which is what we ultimately want. But as the body of individual perturbation-consequence chains grows, they overlap and interweave, and the whole sooner or later must emerge from the sum of the parts (shouldn't it?). The opportunity arises to integrate this information within a mechanistic explanatory mode, an opportunity literally unavailable to biologists until very recently, and we expect this to lead to a fundamental transition in biological thought. Developmental biologists have worked for nearly a century in the perturbation-consequence mode, in which explanation resembles Aristotelian efficient causation. We are on the verge of a shift toward something much more like formal causation; that is, explanation in terms of dynamical formulation and behavior. The first step is to assess where we are with respect to this opportunity. Where in the study of development is mechanism within reach? What do developmental mechanisms look like, and what are the problems associated with identifying or characterizing them? Do we understand any developmental mechanisms well enough to use them as a basis for hypotheses about morphological evolution? The case studies sketched at the end of this essay make us think the situation is quite promising.

Evolutionary biology has come via a different route to much the same point. Evolutionary change in morphology is, literally, heritable variation in development. The developmental mechanism is the fundamental entity whose behavior is altered by mutation, and is thus the substrate of phenotypic variation. Lacking a coherent concept of developmental mechanism and solid examples to consider, evolutionary biologists have had to take for granted the existence of developmental mechanisms that are responsive to selective pressure. The field has historically focused on the nature of that pressure and on interpreting scenarios to account for the existence of specific forms, which themselves are presumed to be more or less "adapted" in aggregate. Thus evolutionary biology employs among its explanatory modes something akin to final causation. Gould and Lewontin ('79) point out in their criticism of the "adaptationist program" that natural selection (Dawkins' "blind watchmaker") is a nearly teleological explanation for phenotypic adaptation, in which possible scenarios for adaptive change are limited only by one's ability to imagine how a surviving outcome can be achieved through a series of selectively favorable intermediates.[3] On the other hand, evolutionary biologists are equally at home with the encapsulation of natural selection in population genetic theory (the watchmaker's apprentice, if you will), which forms the basis for a formal mechanistic mode of explanation. But it's hard to make the leap; without some concrete idea about how genotypes determine morphological phenotypes it becomes difficult to say how allele spread in populations is related to patterns of morphological change.

One of the persistent problems is how we are to explain *patterns* of adaptive morphological change manifest in phylogeny, such as, among other things, missing phenotypes (e.g., eight-legged insects) or parallelisms (e.g., independent evolution of saber teeth in "cats"). More generally, one would like to understand why the adaptive process follows the particular course it does in each instance, rather than another. Most bi-

[3]We wish to be clear that nothing pejorative is intended in this passage. On the contrary, it is only because evolutionary theorists chose to think in terms of selective optima, goals, and adaptationism that evolutionary problems could be spoken about without reference to the nature of developmental mechanism. Certainly during the construction of the Neo-Darwinian synthesis there existed too much debate about the nature of the developmental process for evolutionary theory to consider it explicitly. Moreover, the vibrance and utility of evolutionary theory testify to the validity, if not the completeness, of final causation as an explanatory mode in this context. Finally, despite the critique of adaptationism, the critics do not shift away from a fundamentally teleological viewpoint; introducing terms like exaptation (due originally to Gould and Vrba, '82) or pre-adaptation, does not alter the explanatory model. The anonymous reviewers also drew our attention to two additional points. First, finalistic language may result as much from the prevalence of artificial selection experiments (in which there really is a goal) as from any underlying philosophical dilemma. Second, developmental mechanics, to the extent that it is described by dynamical systems theory, itself requires a good deal of finalistic terminology because of the prominence of stable states, attractors, and limit cycles in the analysis of deterministic systems.

ologists concur that natural selection can't achieve every conceivable goal and that some adaptive routes may be more likely than others. But one encounters difficulty in saying, in a definite sense, whether there is anything more to explain about evolutionary patterns beyond the blind watchmaker. There are bound to be missing phenotypes, because we creatures of the earth can't have had a chance to explore all the possibilities yet, and anyway, what would insects need another two legs for? Furthermore, evolution is a branching process; daughter variants tend to inhabit the same neighborhood of phenotypic space as their parents, so parallelisms may reflect the fact that similar adaptive pressures lead (unsurprisingly) to similar adaptive responses. So it can't be completely clear whether patterns of adaptive change reflect patterns in the selective milieu or reveal constraint.

The word "constraint" reveals the perspective in which this dialectic originated: constrained with respect to what? Clearly, with respect to the ability of selection to accomplish some goal. "Goal" is not far from "purpose," and purpose is final causation. We aren't casting aspersions on evolutionary biologists. Every biologist knows that adapting organisms do not have goals in this sense, that members of an adapting population have no view of the adaptive landscape, and that even if they did they would have no control over the mutational motor that propels them across it. We merely talk about adaptation in this way because it reflects our notion that there exists some ideal response to each selective pressure—the goal.[4] Selection, however slowly, should pull an adapting population quite near such a goal (i.e., to a fitness peak) within the adaptive landscape. There has been a minor rumbling of doubts about the general validity of this assumption. Selection may not, for example, hold a population at an adaptive peak (without incurring some burdensome cost of selection) in the face of high mutation rate. Other doubts arise when one considers the adaptive landscape metaphor: populations could become trapped on sub-optimal peaks, unable to reach the "best" solution without traversing valleys of critically low fitness (Kauffman, '93, chapters 2 and 3).

However, the most debate has been sparked by the notion of *developmental* constraints, the hypothesis that patterns of phenotypic change may sometimes result from the nature of underlying developmental mechanisms (reviewed by Hall, '92). Conceivably, whatever mechanism makes insects bear legs can only make six; maybe the mechanism that makes mammalian teeth has some innate propensity to increase the length of canines. The debate has to do with the difficulty of saying, in any particular case, *whether* an observed pattern is due to constraints or to corresponding selective patterns; in effect, whether a pattern is due to intrinsic or extrinsic factors. We feel this argument misses the point, for it is the case that without even being able to describe developmental mechanics, one can say for certain that *any* mechanism (developmental, electronic, economic, telepathic, whatever) must have describable *variational tendencies*. That is, for any mechanism in the rational universe there must be some set (which could be empty!) of available, functional modifications. This is as true of the most sophisticated mechanisms as it is of the simplest. To make an account of such a set in any specific case, one would need detailed knowledge of the mechanism in question that is well beyond present reach. But we do know some developmental mechanisms as rudiments, well enough to formulate hypotheses about how they could be transformed by mutation. In these cases we can make educated guesses as to how these specific mechanisms might be the substrate for evolutionary change. From this perspective the term "constraint" isn't appropriate. Instead, when we think about morphological evolution in terms of developmental mechanisms, we are really thinking about variational tendencies, of which developmental constraints per se are a special case.

Considering this, we see that this question mark that has traveled so long under the label "developmental constraints" is really the plane of intersection between developmental mechanics and evolutionary mechanics. This question mark is central to the evolution of development. To turn our focus here requires developmental and evolutionary biologists to construct an explicit concept of developmental mechanism and how it functions as the substrate for morphological evolution.

MODULARITY, MECHANISM, AND THE DECOMPOSITION PROBLEM

Whether we start with a decomposition of development into analytic units or with a construction of synthetic units from lower-level entities, we hope to arrive at a conceptualization of building blocks from which could be assembled models of higher-order behavior. There are many ways to identify modules with respect to any particu-

[4]See footnote 3.

lar whole, and in general each different conceptual decomposition of that whole engenders a different set of experiments, a different mode of explanation, and ultimately a different insight. For example, developmental biologists studying the establishment of primary axes within vertebrate limb buds recognize at least two different decompositions of the developing limb. One, based on classical embryology, identifies regions of tissue within the rudiment, such as the progress zone, the zone of polarizing activity (ZPA), and the apical ectodermal ridge (AER), and ascribes to them experimentally defined developmental functions and interactions (Saunders, '48; Saunders et al., '57; Tickle et al., '75; Summerbell, '79). Another, based on genes as abstract units of developmental function, has led to the study of a variety of gene products, including sonic hedgehog (Riddle et al., '93), FGF family members (Martin, '98), and the Hox complex (Nelson et al., '96), that are involved in the establishment of axes within the limb bud. Each of these modes of decomposition has become associated with an accepted type of explanation within mainstream biology, and when we put each class of unit into the pot we hope to get out an explanation of limb development. The more we know within either decomposition, the more comfortable we are taking one as proxy for the other.

Usually, as in this example, units of decomposition are chosen prior to analysis of a given phenomenon or object, for historical reasons, or based on the available tools, or because they are appropriated from existing disciplines. Furthermore, many of the entities familiar in developmental and evolutionary biology (molecules, cells, individual organisms, species, etc.) are obvious in that we readily distinguish them and could articulate why. These obvious entities, defined in more or less concrete terms, constitute the framework of an equally evident hierarchy of biological organization. Yet there are clearly more elusive entities that inhabit intermediate levels within the framework defined by the obvious ones. For example, body parts are decompositional units of animal form, adaptation, and development. Clearly body parts are not as concretely defined as the individual bodies they are parts of or the cells they are composed of. Moreover, anatomy is explicitly hierarchical unto itself, unlike either the cell or the individual; thus we naturally consider a knuckle part of a finger, which is part of a hand, etc.

Consider genes, which are clearly organized into genomes. Empirical experience tells us that there must be some functional organization of genes into higher-order groups *below* the scale of the entire genome, grouped perhaps by the density of interreactivity among gene products. We expect furthermore that any decomposition of the genome in functional terms will be explicitly hierarchical. In this case, however, it remains an operational matter how one chooses to individuate epigenetic modules. One arrives at different criteria analytically and synthetically. Imagine being presented with a map of the epigenetic interactions between all an organism's genes. It should be possible to individuate modules (if they exist) within such a map by connectivity criteria alone: one might adopt the operational definition that a module is any subset with a high internal density of interactions and sparse connection to the rest. This criterion, however, while it suits some applications, doesn't help us integrate molecular genetic data into formalized mechanisms simply because too small a fraction of the total map is yet known. For that problem we need criteria that help us to tell, as we build from the ground up, when we've achieved some degree of "wholeness"; one might adopt module-individuation criteria based on the constitution of an intrinsic behavior. In either case one picks out intermediate entities that are expected usually to fall into a nested hierarchy.

It is such intermediate entities that pose the most difficulty, and with which we would like to associate the term module (as opposed to "unit," which connotes a much more discrete entity than we have in mind). Modules may be more difficult to define in abstract terms and to identify or distinguish from one another in practice than obvious things like proteins and cells, but they are by no means less real as a consequence. Perhaps if we take as a starting point the obviousness of molecules, genomes, cells, and individuals, we can say that we would like to think about intermediate entities that are either composed of or that compose the obvious ones. But here we would run into difficulties as we approach the boundaries. After all, there are well-known problems in trying to define the gene or even the individual in certain contexts (colonial organisms, notably). Paradoxically, our intuition recognizes at least certain modules (body parts, for instance) much more readily than our reason allows; one is tempted to wonder what intuition knows that reason does not, and thus one pragmatic aspect to the decomposition problem involves *a search for rational criteria that accomplish in general what our intuition provides in specific cases*.

There is an intuitive relationship between mod-

ules as defined above and the developmental mechanism: a developmental module is a collection of elements whose intrinsic behaviors and functional interactions yield a mechanistic explanation of an identifiable developmental process or transformation.[5] Having identified a module with its own intrinsic behaviors, one may subject it to further decomposition. Similarly, we expect that modules we treat operationally as isolated entities are coupled to other concurrent modules to effect higher-order entities. Thus it is clear why we should be concerned with the existence and extent of modularity in development: because decomposability is prerequisite to mechanism, the characterization of modularity in development ought to make developmental mechanics more accessible. Furthermore, while it is surely too difficult ever to apprehend the entire logic of development all at once, it may be possible to build such an apprehension through a hierarchical decomposition. As good reductionists, we biologists all hope this to be so or we would never make it to the bench; the extent to which reduction succeeds is related to the degree of identifiable modularity. The point is not to conceptualize the organism or the developmental process as a bag of modules, any more than as a bag of genes, and certainly it will be true that some aspects of development, or some organisms, are more or less modular than others. Nevertheless, the better one can identify logically separable modules, the better paved the road to mechanics.

So far in this essay we have evoked consistently "developmental process" even though it is far from clear that it makes sense to speak of developmental process as if it were a single domain of phenomena in which the modules we want to look for participate. Encompassed under the umbrella of "developmental process" are the epigenetic process (meaning regulated gene expression, dynamic interactions among gene products, cell determination, etc.) and the morphogenetic process (cell shape change, the emergence of physical form). Furthermore, we also have the evolutionary process to consider, and moreover it is often difficult to draw any line between the epigenetic process and plain old metabolism. For each of these reference processes we might expect to identify different compositional units and to find that they enter in different ways into mechanistic explanations. Alternatively we may find that the same compositional units take on different significance when viewed with respect to some alternative process or organization. Thus it is important to investigate how decompositions with respect to different reference processes are related.

THE INTERFACE BETWEEN DEVELOPMENT AND EVOLUTION

If morphological adaptation necessarily means heritable change in developmental mechanisms, how are developmental modules related to units of phenotypic adaptation? One way to operationally define developmental modules is that any subsystem manifesting some quasi-autonomous behavior qualifies. Applying this to the evolutionary process, one decomposition readily presents itself: given a series of ancestrally related forms (i.e., primitive and derived forms plus intermediates), we expect to be able to identify subsets of the shared body plan elements that manifest adaptive change more or less autonomously. We might hope that subsets so characterized would correspond to anatomical elements that we would identify within the individual forms, and indeed this expectation is generally borne out. Instances abound: tetrapod limbs, insect wings, arthropod limbs and segments, mammalian teeth, vertebrate eyes; all are clearly distinct anatomical elements that exhibit specialization autonomous to the body plan of which each is part. Thus, "unit of phenotypic adaptation" means the *continuum* of homologous body parts.

Given such an adaptive unit, we expect an underlying cause in the developmental mechanisms associated with that unit to account for its coherence. We might naively anticipate a one-to-one map between logically separable modules of the developmental process and autonomous body plan elements, remembering that both decompositions are richly nested and hierarchical. One could extend this argument beyond body plan elements to any feature that might conceivably be homologized among a group of related forms, including something as abstract as the dorsal-ventral axis of an

[5] An anonymous reviewer questioned why we focus on the role of modules in explaining things rather than on their role in process per se. We do not know how better to deal with the difficult issue of whether we see modules because they are a useful metaphor or because they are really there, other than to say that it seems useful to think about modules in the context of present knowledge. We don't deny the possibility that once more data are available, modularity (as we describe it) will no longer be the most useful way to think about things. The point here is to say "it looks like it's turning out this way, so let's see where it can get us," so we are not mere idealists concerned only with how we conceptualize things. Still, there is a deep connection between the available data and the metaphors that might be useful to describe those data. Thus, the notion of the gene as determinant flourished when single genes unconnected to each other constituted the available data, and the metaphor of the developmental pathway has been useful as long as most of the available data fit into linear chains. To a certain extent the issue becomes moot because our saving grace, as scientists, is that sooner or later we junk explanations and metaphors if they don't fit the facts.

animal or a particular arrangement of cell types in a tissue. Indeed, when we ask if there is such a one-to-one relationship, we tend to find that there is: thus the arthropod segment has its segment polarity genes, the vertebrate axial complex has its Hox genes, and the tetrapod limb has its panoply of hedgehog, patched, BMPs, FGFs, wnts, and (again) Hox genes.

Since it seems clear that within an individual species we might expect the modules of developmental regulation to correspond, at least often, to modules of phenotypic adaptation, we must question the extent to which these are phylogenetically stable associations. The easy answer is to cite examples, of which there are many, that prove that such relationships are not (always) stable. Instead we would like to explore why this is likely to be generally true, because it has important consequences for how we study the evolution of development and how we interpret data. To frame this problem more clearly, it is useful to contrast development as a process with evolution; there are at least these two reference processes of interest. Biologists view development as a collection of dynamic processes arranged hierarchically in space and time that collectively produce from some well-defined initial state (the fertilized egg) an adult organism (i.e., an equally well-defined specific collection and arrangement of anatomical parts). We tend to associate with each subprocess some landmark end state (e.g., establishment of primary axes, allocation of an organ rudiment, etc.) or, more generally, a transformation of embryonic state. While we can study such individual processes in conceptual isolation, they are coordinated in space and time to produce a harmonious whole. For each such process we seek to identify a set of parts—a module—whose intrinsic properties and interactions collectively determine, given appropriate initial and boundary conditions (which represent coordination with other subprocesses), the mechanism underlying that dynamic transformation. Here the focus is primarily on identifying the specific properties and arrangements of parts plus the specific initial and boundary conditions that get the job done—within an individual organism.

By contrast, evolution is an interplay between natural selection (Dawkins' "blind watchmaker," who cares little for design as long as "it works") and the processes by which variants arise and distribute (what we called the "watchmaker's apprentice" earlier in this article). Phenotypic variation is filtered by natural selection into a specific history of change that would likely be somewhat different were it possible to repeat it even under the same external conditions. One can distinguish conceptually between processes that determine specific patterns of phenotypic change within natural populations given the range of available heritable phenotypic variation, and processes internal to embryos governing the production of that variation, (i.e., that determine the structure of the genotype-phenotype map [*sensu* Wagner and Altenberg, '96]). The present discussion is concerned mainly with the latter type of process, the internal production of heritable phenotypic variation and the ways in which this defines possibilities for adaptive evolutionary change. The same decomposition described above seems natural, based on logically separable parts or processes and their associated developmental mechanisms. But in this case we are not interested in specific instances of mechanism with respect to a specific part or process within an individual organism, but rather in the variational properties of developmental mechanisms with respect to genetic mutation. That is, we are interested in the range of variation that genetic mutation could produce in the properties of a given developmental module or mechanism given both its current structure and the ways in which coordination with other modules determine which variants might "work". Given a mechanism at work in some context, in what sense is it a substrate for evolutionary change? What can it be made to do?

Now we must face the fact that *the criteria determining which variants persist have, in all likelihood, little to do with the preservation of any particular relationship between mechanism and function*. The evolutionary process doesn't necessarily care so much about exactly how the dorsal-ventral axis is set up, the tooth anlage is specified, or the neural tube formed, as long as the end state is equivalent. Consider a trivial example: imagine a signal transduction pathway in which a cell-surface receptor activates a kinase, which then activates a target. Now imagine introducing another protein kinase, and that this kinase can be activated by the receptor and in turn activate the appropriate target, and moreover do a better job, somehow, than the original. Couldn't the evolutionary process junk the original in favor of the newcomer?

Surely if we can imagine this scenario with a single gene product, we can also imagine an entire module, an entire mechanism, being subsumed should something better arise to take its place. Moreover, what if we begin with a very so-

phisticated mechanism, say one that patterns an entire organ rudiment; such a sophisticated mechanism might readily be broken down into many modular parts: perhaps a few signal transduction cascades, several gene regulatory switches, etc. Might we not imagine that each of these parts could be successively replaced, even several times over, in a genetic version of continual xenoreplacement? This may sound far-fetched, but consider one notable example: there can be little doubt that the segmentation of long-germ and short-germ insects is homologous in a rather deep sense. In *Drosophila* we know how segments are laid out, and this mechanism depends on the fact that it takes place in a syncytium (reviewed in Lawrence, '92). No such mechanism could possibly make segments in short-germ insects, where all but the first few segments are patterned as they proliferate from a growth zone. A massive replacement of developmental mechanism has taken place, and the evolutionary intermediates may be obscure.

This roundabout approach is intended to expose a challenge that arises at the interface between developmental and evolutionary mechanics. The implication is that we will only rarely, if ever, be able to pin down any stable complex of developmental mechanism and phenotypic characters that behaves as a discrete unit with respect to both the evolutionary and developmental process. Despite a few attention-grabbing examples, *there is no a priori reason to believe that the same instantiation of a developmental mechanism underlies a conserved developmental process in even closely related organisms*. Indeed, empirical observation confirms that this need not be so (see Félix, '99; see examples in Roth, '88; also Table 1 in Wagner and Misof, '93). These considerations reveal grave difficulties for applying any of our classical comparative tools, particularly our classical notions of character identity, to the evolution of development. One principle goal in evolutionary biology is to reconstruct the actual history of phylogenetic change, and a central problem of phylogenetics is the identification of homologous characters and determination of polarity of evolutionary change in those characters. Many current workers, inspired by the apparent conservation of a few better-known developmental mechanisms, are eager to use developmental mechanisms (or at least spatial regimes of gene expression) as quasi-phylogenetic tools, especially as diagnostics for homology (for example see DeRobertis and Sasai, '96; Arendt and Nübler-Jung, '99). Clearly one property that a unit of phylogenetic change must possess is at least some degree of persistence over evolutionary history, but how could we trace such a scenario as described above? We have developed a hypothetical nightmare in which a putative phylogenetic unit undergoes continual xenoreplacement at the molecular level; of course the corollary is that the replaced parts are probably not being junked, but rather are recycled into another context, to replace another module in turn. The more dissociable (i.e., the more modular) a mechanism, the more obscure will be its phylogenetic origins and its correspondence to body plan elements. If we take modularity at all seriously, then any attempt to use developmental mechanisms as phylogenetic tools is doomed: how could one hope to distinguish between bona fide conservation (a stable history between mechanism and character) and re-use or (worse yet) re-invention?

An inversion of perspective makes the situation look much more promising, and a colleague of ours has put it best:

> "...the character [identity] problem and the homology problem have been primarily discussed over the generations from a taxonomic and comparative point of view; but I think this is completely wrong-headed and most of the baggage we are dealing with here is coming from this history. Characters do not exist to please taxonomists, [just as] genes do not exist to please geneticists; rather the fact that we can identify and work with genes tells us something about the functional and physical organization of the genetic material. In the same sense, the fact that we can identify homologous body parts tells us something about how the phenotype is organized. I would like to look at characters and homologues from this biological point of view, rather than the perspective of how to recognize them and how to use them to reconstruct phylogeny, because that's not the reason they exist; that's not the mechanistic problem that we have to solve." *— G. Wagner, speaking at FHL modularity workshop, '97*

Rather than view dissociability as a problem for comparative biologists, let us recognize it as an architectural feature of evolvable developmental systems, a feature whose origins and consequences deserve attention. Over the years, authors too numerous to cite have noticed that, indeed, it is difficult to imagine evolution of morphological diversity as we know it without dissociability. Furthermore, the litany of well-known evolutionary transforma-

tions, including homeosis, all types of heterochrony, adoption of direct development, and so on, literally imply dissociability either among body plan elements, among developmental mechanisms, or both.

CHARACTER IDENTITY, HOMOLOGUES, AND VARIATIONAL TENDENCIES

Nevertheless, we need ways to assess character identity. In the case of body parts, for example, we need rational criteria that will generalize for us what our intuition does so well in particular cases. To our knowledge the most useful account of character identity for EvoDevo is the "biological homology concept" developed by Roth ('88, '91) and Wagner ('89a,b, '95), and our gloss is based on their work. Let us begin by asking, what does our intuition do? Wagner ('95, citing McKitrick, '94) notes that we recognize homologues based on parsimony: given a character and a choice between candidate homologues, we choose the correspondence that requires the least transformation. To borrow Wagner's illustrative style, it is easier to think of the bird's wing "becoming" a dinosaur's forelimb than to think of the wing becoming, say, the tail.[6]

Wagner ('95) notes that *this parsimony approach makes an implicit statement about the variational tendencies of the characters in question*. We cannot imagine how natural variation could transform a bird wing to a dinosaur tail, but we *can* see how natural variation could transform it to a dinosaur forelimb, therefore we conclude that the bird wing and the dinosaur forelimb are manifestations of the same homologue. We have therefore already assumed not only the existence but also the *evolutionary relevance* of what are classically called developmental constraints! Now we have come full circle: we began the previous section with an intuitive decomposition of animal form into units of phenotypic adaptation; we equated a continuum of homologues with such units; the unit, in reference to the adaptive process, is manifest within the continuum of variant forms; and now we see the unit must in fact *be defined by its variational tendencies,* for it is only those tendencies that maintain the identity of a character through the adaptive process. We cease (or *should* cease) to identify homologues when we can no longer imagine transformation by naturally-arising, heritable phenotypic variation. Moreover, *if we ask how phenotypic characters participate in the evolutionary process, it is through the variety of possible forms determined by the variational tendencies of each character*.

Whence variational tendencies? Obviously, when we are talking about morphological characters, variational tendencies come largely from developmental mechanism, whatever it is. This is paradoxical: there is no inherent stability to the genotype-phenotype map, no one-to-one gene- or developmental process-to-character relationship, yet we confront the widespread existence of stable patterns whose coherence must be embodied causally within a shifting sand of developmental process beneath it. Roth ('88, '91) and Wagner ('95) conclude that the intrinsic stability of homologues amidst the evolutionary process is an emergent property that transcends the (possibly rapid) evolutionary transformation of the processes which form them.

There is a naive way out of this conundrum. If most of the time organisms experience stabilizing selection with respect to most characters, then if a variant were to arise in which one module of a particular developmental mechanism were to be replaced by another that performs equivalently, this replacement could be selectively acceptable. So we get a little bit of change in the developmental mechanism, and none in the character it produces. Functional constraints impose conservatism at the level of phenotypic units, while allowing some flexibility in the developmental process. This explanation, while plausible, is so mundane as to merit no further attention. Also, it assumes an extreme degree of modularity among developmental processes that is unlikely to be general enough to explain the origins of character stability in more than a few instances.

Wagner and Misof ('93), inspired by experiments on regeneration in blennie fin rays, suggest an interesting possibility. They distinguish between generative mechanisms (like rudiment specification, pattern formation, and morphogenesis) and morphostatic mechanisms, which include any mechanism involved with maintenance of the organ or structure in question during ontogeny. A trivial example would be a mechanism that maintains the relative distribution of cell types in a growing organ. Regenerative processes certainly reflect the existence of morphostatic mechanisms,

[6]A flip side to this, though perhaps obvious, deserves emphasis: it is an important criterion that it be easier to draw a correspondence than it is to postulate novelty. Thus, we have to convince ourselves that it is easier to transform the reptilian forelimb to a bird wing than it is to first dispose of the forelimb and then invent a wing. There is little danger of forgetting this criterion in the case of complex body parts such as limbs, but there is much danger in the case of simpler "characters" such as differentiated cell types or developmental mechanisms, to say nothing of attempts to use the expression of individual genes such as *Distal-less* (for example, Panganiban et al., '95, '97) or Brachyury/T (Kispert et al., '94) as homology markers.

because to account for regeneration there must be some regulatory mechanism that senses incompleteness and activates wound healing, blastema formation, etc. and then turns it all off again when completeness is again achieved.

Clearly, morphostatic mechanisms could buffer against variation in generative processes. There may be a wide array of functional forms that variation in generative processes could manifest, but a morphostatic mechanism could attenuate that variability. On the other hand, a character could not be buffered in the same sense against changes in morphostatic mechanisms. Unless the variability of generative mechanisms is insufficient to fill the space of possibilities allowed by morphostatic variability, variation in morphostatic mechanisms should correspond to variation in the phenotype. Both classes of mechanism therefore determine variational tendencies, but Wagner and Misof argue that because morphostatic mechanisms can impose an outer limit in the space of possible variation, they may be primary determinants of the continuity of homologues in the face of changing generative processes. Thus, phenotypic characters may be endowed with a certain degree of conservatism by developmental constraints, again while allowing some variation in generative mechanisms.

The conceptual division of developmental process into morphostatic and generative modes has other interesting implications. First, the notion of morphostatic mechanisms may help explain cases of "bottlenecking" in the evolution of development. For instance, the generative processes that lead to segmentation differ radically between short- and long-germ insects, yet both types of development lead to a primitively-segmented embryo with similar neurogenic and appendage-making patterns in corresponding segments (Patel et al., '92). The segment polarity genes, which likely function similarly in short- and long-germ insects (Nagy and Carroll, '94), may be among the morphostatic mechanisms that maintain the identity of segments in insects. Our own efforts to simulate the dynamic behavior of segment polarity genes have shown that even a small subset of known interactions can stably maintain an asymmetric segmental pattern in a field of cells (von Dassow, Meir, Munro, and Odell, in preparation).

Second, under certain conditions morphostatic mechanisms might channel otherwise aberrant generative variation into useful functional variation. The autopodium of the amniote limb can generate more than the usual five condensations (six-and seven-toed cats, the occasional polydactylous human); some sort of mechanism evidently is able to convert the extra rudiments into usable digits. Somewhat more extreme is the recent finding that metamorphic brittle star pluteus larvae can drop off fully-formed juveniles, swim back into the plankton, regenerate, and form a new juvenile (Balser, '98). Starfish larvae also have remarkable regenerative capacity (Minako Vickery, personal communication), and Jaeckle ('94) demonstrated that starfish *larvae* can reproduce asexually by budding. Thus, morphostatic mechanisms (of which regenerative powers are a manifestation) can open up entire life history variations. They do not always impose the outer bound on the space of possibilities, but may even facilitate hops through that space.

We do not pretend herein to solve everything by applying conceptual distinctions and imagining the consequences. Rather, the point we wish to make is that the problem of correspondence between *natural units of developmental process* and *natural units of evolutionary process* is neither trivial nor presently soluble. The existence of the problem tells us first, to turn our attention to exploring the nature and origins of variational tendencies in developmental mechanisms; second, to ask what systems-level properties emerge from conspiracies of lower-level entities; third, what can we learn about what makes developmental architecture evolvable; finally, how did we get such an architecture in the first place?

A FEW INSTRUCTIVE EXAMPLES

We present a selection of case studies that illustrate some of the concepts to which we want to draw attention here. These examples are not presented to prove any particular point, but rather because each of these cases is an exceptional opportunity to study the evolution of developmental mechanisms in relation to phenotypic adaptation, using molecular and comparative approaches together.

Hedgehog, patched, and company: an epigenetic module

First we discuss a simple example of a tiny epigenetic network that appears to behave evolutionarily as a module. This example is afforded by recent studies of the regulatory interaction between the *Drosophila* segment polarity genes *patched* (*ptc*) and *hedgehog* (*hh*) along the anterior-posterior compartment boundary in *Drosophila* imaginal discs. In the posterior, *engrailed* (*en*) promotes *hh* transcription (Tabata et al., '92).

ptc is active in the anterior compartment and is repressed in the posterior compartment by *en* (Hidalgo and Ingham, '90). However, *ptc* is transcribed only at very low levels in most cells of the anterior compartment because the Ptc protein, when active, leads via a subtle interaction with the transcription factor encoded by *cubitus interruptus* (*ci*) to the repression of *ptc* transcription (Aza-Blanc et al., '97). The secreted protein encoded by *hh* binds to and titrates away Ptc, thus relieving transcriptional repression of *ptc*; this allows accumulation of Ptc adjacent to the posterior compartment, where *hh* is expressed (Marigo et al., '96). Wherever *hh* is expressed, neighboring cells express *ptc* unless something else (such as Engrailed) prevents *ptc* transcription.

hh, ptc, ci and their associates interact in such a way as to constitute a tiny, re-usable epigenetic module. The re-usability of this module has been demonstrated strikingly in vertebrates. In the mouse, *hedgehog*-like genes are expressed in a variety of tissues including notochord and floorplate, limb bud, gut epithelium, whisker buds, nasal placode, and more; in every case the mouse *patched* is expressed in cells immediately neighboring *hedgehog*-expressing cells (Goodrich et al., '96). Since many of these structures are evolutionary novelties, one cannot help but consider this a manifold example of reuse. Whatever property makes this tiny module useful, it is abundantly clear that it is not common ancestry but common biochemistry that unites the long list of embryonic structures in which *hh* and *ptc* are expressed in complementary patterns.[7]

One of the best-studied roles of the *hh*/*ptc* module is in the formation of parasegmental boundaries and polarization of segments in *Drosophila* embryogenesis. They were originally identified as segment polarity genes, a class that includes genes involved in Hh signaling (*fused*, *smoothened*, *costal-2*, and others; for review see Kalderon, '97), *en*, *wingless* (*wg*), and genes like *porcupine*, *armadillo*, and *disheveled*, whose products participate in Wg signaling (reviewed in Cadigan and Nusse, '97). In the row of cells posterior to the parasegment border, as in imaginal discs, En promotes *hh* transcription (Tabata et al., '92). Hh tirates away Ptc, consequently altering the relative abundance of repressor and activator forms of Ci, such that the activator accumulates in cells just anterior to the boundary (Aza-Blanc et al., '97). One Ci target is *wg*; full-length Ci activates *wg* anterior to the boundary (Von Ohlen and Hooper, '97). Wg is required for *en* expression in cells posterior to the boundary (Vincent and O'Farrell, '92), and thus there is a closed loop of interactions that, it has been hypothesized, stably maintains the boundary throughout development. This hypothesis implies that the *hh*/*ptc* module has been incorporated, in this instance, into a higher-order network that exhibits a more complicated intrinsic behavior, namely the stable spatial regime of segment polarity gene expression.

As mentioned previously, the segmental organization of insect embryos seems conserved throughout insect evolution, including the manifestation of parasegments (for example, Nagy et al., '91), the mechanism of limb specification (Panganiban et al., '94; Warren et al., '95), and the function of at least some of the segment polarity genes in maintaining the compartment boundary (Nagy and Carroll, '94). Yet segment specification differs radically among insects, and it is impossible to imagine that the same molecular mechanisms are at work in every case (Patel et al., '92; Patel, '94; Grbic et al., '96; Ho et al., '97). These observations led us to hypothesize that the segment polarity genes must be a module according to the following strong definition: a module has a characteristic intrinsic behavior in the absence of any specific, persistent, exogenous influences on its components, and it may be triggered to express this behavior through a small number of (one or a few) generic inputs. We set out to test whether or not this is the case by building a dynamical simulation of the segment polarity network. In this case, the requisite behavior seems to be the asymmetric, segmentally reiterated co-expression pattern of *wg*, *en*, and *hh*, all of which function as "outputs" of the segment polarity gene network.

We found that a surprisingly small subset of known segment polarity gene products and their known interactions (as characterized in *Drosophila*) can satisfy these criteria for modularity (von Dassow, Meir, Munro, and Odell, in preparation). That is, the skeleton of the segment polarity gene network can stably maintain the appropriate expression patterns for *wg*, *en*, and

[7]Indeed we can't even be sure we're talking about re-use or reinvention here. It is no more "homologous" that *hh* and *ptc* are coexpressed in two different tissues than it is that serine, histidine, and aspartate are found at the active site of both subtilisin and chymotrypsin family proteases. Serine, histidine, and aspartate are not the only way to cleave a peptide bond (many other proteases exist), but it does not nowadays surprise us much that two otherwise unrelated proteins should have come upon the same three-dimensional arrangement of amino acids, and that, given such an arrangement, both proteins act as proteases. By analogy, *hh* and *ptc* are probably not the only way to do whatever it is that they do, but we should not be surprised if two otherwise unrelated tissues have employed them for that function.

hh in a two-dimensional field of cells without any exogenous influences *given a suitable choice of kinetic parameters*. Moreover, our simulation is surprisingly insensitive to both initial conditions and the choice of kinetic parameters. Although there are about 50 free parameters, each of which might vary over several orders of magnitude, an astounding one in 200 *randomly chosen* parameter sets leads to the proper behavior, and it is typical to find that the model is insensitive to variation in individual parameters over a range of 2- to 100-fold or more! Thus, we believe we have shown that the inherent modularity of the segment polarity network could explain how insect embryos possess homologous segments despite radically different means of segment specification.

The evolutionary lability of nematode vulval development

Recent studies of the evolution of vulval development in worms reveal a surprisingly rapid evolution of developmental process despite overall conservatism in adult morphology; Félix ('99) reviews these examples in detail. In *C. elegans*, two cells (Z1.ppp and Z4.aaa) interact symmetrically to decide which will become the anchor cell and which the ventral uterine precursor. This interaction is mediated by a feedback loop involving the *Notch*-like receptor *lin-12* and its *Delta*-like ligand, *lag-2*. In *C. elegans* Z1.ppp and Z4.ppp form an equivalence group because in half the animals one cell forms the anchor cell, and in half the animals the other cell does so. In other nematode species, these two cells are not equivalent but still constitute a competence group; both cells can form the anchor cell, but usually Z4.aaa does so. In still other species, Z4.aaa always forms the anchor cell but Z1.ppp can do so in the absence of the normal precursor. Finally, there are nematode species in which the anchor cell and the ventral uterine precursor are each specified autonomously (reviewed in Félix, '99). Based on causally associated changes in gonad morphology, Félix (personal communication) regards the equivalent condition, as in *C. elegans*, as primitive. Thus we have a continuous series in which the differentiated character is preserved (namely, the presence of the anchor cell and the ventral uterine precursor); in all likelihood the molecular mechanism is to a certain extent conserved (otherwise we might not see such a continuous series), and yet the nature of the developmental process has completely changed. Indeed, this case illustrates conversion of a regulative to a mosaic process.

The anchor cell participates, at least in some nematodes, in the induction of the vulva. In *C. elegans*, six cells (P[3–8].p) arrayed along the AP axis of the animal may participate in vulva formation. The anchor cell is normally closest to P6.p; it secretes a signal that is received in highest concentration by P6.p, and in lower concentration by the two neighbors. P6.p thus adopts the central vulval fate (1°), its neighbors adopt the outer vulval fate (2°), and the remaining three cells do something else entirely (reviewed in Félix, '99). There are at least two other regulatory processes in *C. elegans* that influence vulval cell fate decisions in this population of cells. First, the cell (P6.p, normally) that receives the most signal from the anchor cell inhibits its neighbors from adopting the 1° fate. According to Félix (personal communication) it is not clear in *C. elegans* whether this lateral inhibitory mechanism or the anchor cell signal predominates in normal development. Second, the six vulval precursor cells are not uniformly competent; the expression of the Hox complex gene *mab-5* makes P7.p and P8.p somewhat refractory to induction. Sommer and Sternberg ('96) and Félix and Sternberg ('96, '97) have characterized development of the vulva in six nematode species, and found surprising variation in the relative role of these different regulatory interactions. In some species the primary mechanism for vulval fate specification seems to be restriction of competence; other species have varied the timing of inductive events relative to division of the precursor cells; in one the induction from the anchor cell seems to have been dispensed with entirely. Yet apparently only in the last instance is the change in the mechanism of fate specification associated with a morphological alteration, and in only one other species is there any change in the contribution of precursor cells to each vulval fate.

In the case of cell fate specification among the P(3–8).p cells, the evolutionary variability in developmental mechanism is explained by the fact that nematodes have a kit of at least three mechanistically distinct but functionally overlapping processes that contribute to the specification of vulval cell fates in this population of cells. The evolutionary series characterized by Sternberg, Félix, and their colleagues is based on altering the relative weighting of these mechanisms. In the case of anchor cell determination, there is as yet no reason, as far as we are aware, to think that there is more than one basic mechanism at work, though this is a possibility. Instead, one hypothesis is that this one mechanism, based on the *lin-12* signal-

ing pathway, experiences different contexts in each species; perhaps the initial conditions differ or perhaps exogenous inputs modulate the response of one or the other cell. Strikingly, exactly such variation in the function of *lin-12*-dependent fate specification mechanisms exists within *C. elegans*: while this molecular mechanism mediates a symmetric interaction between the anchor cell precursors, at other times and places in development it mediates biased interactions, inductions, and autonomous specifications (Félix, '99).

In both cases the conservatism of the end state could come from functional constraints alone, with the evolutionary process oblivious to these sorts of changes in developmental process as long as "it" (the anchor cell or the vulva) still works. In neither case is it clear whether changes in the developmental process are selected according to the changes in morphology they are associated with, or if they are completely independent of it. As Félix ('99) observes, it's possible that the observed evolutionary changes in developmental mechanism are nearly neutral. If so, it is facilitated by the modularity of the developmental process.

Adaptation of insect segments

So far, we have discussed dissociability between morphological characters and developmental processes from the perspective of the character, making it seem as if characters are stable and developmental processes come and go. Of course characters come and go as well, and some developmental processes may be remarkably stable. The role of the insect Hox cluster in segment identity is an important paradigm. Early on, there was the notion that insect segmental diversity was due to the invention of new homeotic genes (Akam et al., '88). Starting with a myriapod-like animal, a new gene might arise to suppress appendage formation in the abdomen. Perhaps another new gene distinguished legs from antennae; and more recently, perhaps the origin of two-winged from four-winged insects involved invention of a wing suppressor gene active in the metathorax. With the discovery that genic diversity of the Hox complex has been essentially conserved since well before the origin of insects (reviewed by Carroll, '95), the relevance of this hypothesis in insects is minimal at best.

Instead, perhaps the expression domain of Hox genes changed. This turns out not to be the rule either; numerous reports demonstrate that expression domains of Hox complex genes are rather stable evolutionarily. For instance, in *Drosophila* *Ubx* selects between wing on the mesothorax and haltere on the metathorax. Yet *Ubx* expression has barely changed since the dawn of the insects: not only in four-winged insects (Kelsh et al., '94) but even in apterygotes (Carroll et al., '95), a *Ubx*-like gene is expressed in the metathorax. Thus, changing boundaries of *Ubx* expression can't be responsible for reduction of the hindwing in Diptera. The expression domains of *abd-A* and *Scr* also seem to be similarly stable (Tear et al., '90; Rogers et al., '97). These two Hox genes repress wing primordium formation in *Drosophila*. Yet there is good reason to believe that wings initially arose on all trunk segments (Kukalova-Peck, '78), despite expression of *Scr*-like genes in the dorsal base of the prothoracic limb (Rogers et al., '97) and *abd-A*-like genes in the abdomen (Tear et al., '90). Thus it must be the case that the wing-forming mechanism acquired an input from each of these Hox complex genes at some point in evolution, definitely after the invention of wings. Nor can *Ubx*- or *abd-A*-like genes have an ancient role in limiting limbs to the thorax, since Averof and Akam ('95) have shown that in crustaceans both are expressed throughout the leg-bearing region. In *Drosophila* and butterflies these genes repress *Distal-less*, which is part of the trigger for limb primordium formation (Warren et al., '95). Again, the limb-forming mechanism must have acquired this input after the origin of arthropods. Looked at the other way around, the Hox genes are part of an evolutionarily stable mapping mechanism in insects, and as a ready source of positional information they have progressively insinuated themselves into a position of control over morphogenetic modules like wings, limbs, and other organs.

The Hox complex does not have quite the same evolutionary role in other notable groups, like the crustaceans and the vertebrates. Hox gene expression boundaries *do* shift relative to each other and relative to the major body regions in the crustaceans, and these shifts may be involved in appendage diversification (Averof and Patel, '97). A similar phenomenon has been demonstrated in the vertebral column of amniotes, although in this case the Hox complex appears to retain a causal link to the distinctions between body regions; the number of segments in the major body regions and the detailed morphology of segments seems to change along with changing Hox expression boundaries (Burke et al. '95; reviewed by Carroll, '95; and by Müller and Wagner, '96). The derivation of the tetrapod limb from the fins of fishes provides yet another example of a complex rela-

tionship between Hox genes and morphological evolution, in which an anchored Hox expression domain may have been elaborated into the novel autopod (reviewed by Müller and Wagner, '96).

The vertebrate limb I: an epigenetic trap

The vertebrate limb bud affords an example of a developmental constraint—a systems-level property that limits variability (reviewed in Martin, '98). In the limb bud there are three classically defined territories: the apical ectodermal ridge (AER), the zone of polarizing activity (ZPA), and the progress zone. The progress zone consists of proliferating cells that fuel the growth of the limb; the ZPA consists of mesodermal cells that produce a signal that specifies the anterior-posterior (AP) axis of the limb; and the cells of the AER signal to the progress zone to continue proliferating (Saunders, '48; Saunders et al., '57; Tickle et al., '75; Summerbell, '79). The most important signals produced by the AER and ZPA are FGF-4 (Niswander et al., '93) and sonic hedgehog (Riddle et al., '93), respectively, and the combined action of these two signals maintains the activity of the progress zone. Also, sonic hedgehog triggers pattern formation along the AP axis. However, to respond to sonic hedgehog, cells in the limb bud need to see FGF-4 from the AER. Furthermore, activity of the AER depends on sonic hedgehog. Thus, there is a positive feedback loop between the AER and the ZPA that is causally relevant to the growth of the limb bud, and there is also an equally important requirement for both signals in the axial patterning of the organ (Laufer et al., '94; Niswander et al., '94). This double dependence imposes a constraint; we quote Wagner, who explained it to us:

> "[in this instance] pattern formation and (thereby) constraints on variation are mechanistically linked to the very existence of [the limb]... So constraints [arise] because you cannot fiddle around with the existence of anterior-posterior polarity without endangering the very existence of the whole limb. In this type of system a constraint comes into play when the same genes are involved in some aspect of pattern formation and in the very existence of the organ... the evolutionary process has gotten into an epigenetic trap; it's trapped into producing something that is polarized or not producing it at all." — *G. Wagner, speaking at FHL modularity workshop, '97*

This is a terribly simplified sketch of the developmental mechanism at work in the limb bud, but we can expect that a fuller account would reveal many more interdependencies of this sort (some of which are indicated in Martin, '98). Such interdependencies may be commonplace.

II: urodele versus anuran and amniote limbs

We continue with another vertebrate limb example that illustrates how variational tendencies serve as homology criteria. The tetrapod limb consists of three units, of which only the hand is unique to tetrapods. Although anatomically urodele and anuran limbs are fairly similar, developmentally the urodele hand differs from the anuran or the amniote hand (reviewed by Wagner et al., '99). The urodele limb has no morphologically distinct AER. Urodele fingers grow out as a bud from the developing hand, whereas the anuran and amniote hand is formed by remodeling a paddle-shaped rudiment. Anuran and amniote digital condensations form in a different order from urodele digits; the anuran/amniote hand begins with the fourth digit, then three, two, and one, concomitant with the fifth, whereas urodele fingers develop one through five. Wagner et al. ('99) found that Hox expression domains in the urodele limb are significantly different from the anuran/amniote limb. The urodele and anuran/amniote hands also have different variational tendencies: the anuran/amniote hand tends to retain primarily the fourth finger when it loses digits in either phylogeny or under experimental conditions, whereas the urodele hand tends to retain digits one and two.

If we take seriously the notion that homologous characters are united during adaptive processes by their innate capacity to produce *characteristic* heritable phenotypic variation, then urodele and anuran/amniote hands are different things and we oughtn't consider these two limbs strictly homologous. After all, one criterion for homology is that we must convince ourselves that the hypothesis of independent origin is less parsimonious than the most reasonable hypothesis of sameness. Each type of hand has distinct properties that unite instances of it and distinguish it from the other, and it is impossible to see how the variational tendencies of one type of hand could encompass the other type. Are urodele hands a separate invention from anuran hands, which happened to co-opt the mechanism used to determine the morphological characteristics of digits? That seems unlikely since the living amphibia are monophyl-

etic. Is this a case in which the generative process differs but morphostatic processes preserve form? This would be surprising since the developmental processes responsible for the anuran/amniote limb don't seem intrinsically labile; from what little we know of these processes at the molecular level, they have been conserved since before the divergence of amniotes from the amphibia.

Wagner and colleagues suggest two more plausible scenarios. In the first, they note that fossil forms at the base of the tetrapods have eight-fingered hands. Perhaps the urodele hand is derived from the posterior five digits of the ancestral eight and the anuran hand was derived from the anterior five. This would mean that the most stable digit (the fourth-most-anterior in anurans and amniotes, the anterior-most in urodeles) is actually the same in each group, corresponding to the fourth digit of the ancestral eight-fingered hand. This doesn't explain differences in digit morphogenesis, but it explains the difference in the order of development and in which digits tend to be lost. Second, Wagner et al. ('99) point out that among urodeles are a number of groups with a strong tendency to drastically reduce digit number. Perhaps the urodele hand is not a separate invention, but a re-invention after reduction; the urodeles re-invented additional digits out of functional necessity but there was no reason to place them anteriorly or posteriorly, or develop them by budding or not. Neither scenario accounts for the fact that on the basis of adult morphology comparative anatomists have been comfortable identifying the five digits of the anuran, amniote, and urodele hands as I, II, III, IV, and V in each group. In the first, we would have to posit that the urodele hand, once the posterior five digits had been culled from an eight-fingered ancestor, underwent a homeosis so that the original fourth digit (the new first digit) adopted adult morphology of the first digit. In the second, we would have to expect that the mechanism responsible for specifying digit identity could be anchored by the one or two ancestral digits, then re-adopted by newly reinvented ones.

III: dissociability within the limb

In a related phenomenon, the tetrapod limb provides an excellent example of dissociability among developmental mechanisms. One long-standing paradox has been the homology of the forelimb digits of birds, which may have been resolved by Wagner and Gauthier ('99); their solution assumes the dissociability of some of the morphogenetic mechanisms that shape the limb. Anatomically the forelimb digits of birds correspond to digits I, II, and III of the reptilian hand; this derivation is evident in the lineage of theropod dinosaurs from which birds arose. Developmentally, the digits of birds ought to be II, III, and IV. As noted previously, when amniotes (which ancestrally possess five digits on each limb) undergo evolutionary reductions in digit number, they "invariably" (Wagner and Gauthier, citing Morse for "Morse's law") lose the first, then the fifth. During amniote limb development, the fourth primordial finger forms first, then three more condensations, in a line with and connected to the fourth, form successively more anterior (Alberch and Gale, '85). The thumb is the last to form on this "metapterygial" axis. The fifth condensation appears posterior to the fourth concomitant with the elaboration of the metapterygial axis. In birds, the forelimb develops four digital condensations in the autopodium, three of them along the metapterygial axis. These are therefore identified embryologically as digits II, III, and IV. The fourth condensation in bird forelimbs is identified with digit V, and is resorbed before differentiation of the hand. In addition, experiments with mitosis inhibitors show that the thumb is most sensitive. Thus the paradox is: how can birds have an anatomical thumb when they have no embryological thumb?

To resolve the paradox, Wagner and Gauthier propose that the developmental process that makes the digital condensations is causally independent from the "ensuing developmental individualization of those repeated elements as they become the functional fingers in the mature hand." If this is so, then they suggest it is possible that in the evolution of what eventually became the bird wing a frame shift (their term) took place that associated the condensation originally destined to make digit IV with the morphological fate of digit III, condensation III to morphology II, and condensation II to the thumb. They support their hypothesis on the basis of Hox gene expression patterns in the fore- and hindlimb of chick embryos, which reveal the requisite frame shift. Thus, Wagner and Gauthier propose a phylogenetic scenario in which the ancestors of birds, the theropod dinosaurs, faced a conflict between a developmental constraint that favors loss of particular condensations and a functional requirement for a thumb, and that the evolutionary route through this conflict was facilitated by the dissociability of the differentiative process that confers adult functional morphology on the digits,

and the morphogenetic process that provides the primordia.

The reason we choose this example to close our essay is that there seems to be no way to resolve the paradoxes that the tetrapod limb presents without many of the conceptual tools for which we have been advertising here. Other scenarios than the one advocated by Wagner and Gauthier are certainly conceivable, but as far as we are aware all of them involve some point of dissociability in the developmental process that forms the tetrapod hand. To solve this particular riddle, and so many others like it, we need to believe in developmental constraints and we must hypothesize modularity and dissociability. It remains, in every case, for us to explore the mechanistic origins of these properties.

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