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Plasticity and Constraints in Development and Evolution

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ABSTRACT Morphological similarities between organisms may be due to either homology or homoplasy. Homologous structures arise by common descent from an ancestral form, whereas homoplasious structures are independently derived in the respective lineages. The finding that similar ontogenetic mechanisms underlie the production of the similar structures in both lineages is not sufficient evidence of homology, as such similarities may also be due to parallel evolution. Parallelisms are a class of homoplasy in which the two lineages have come up with the same solution independently using the same ontogenetic mechanism. The other main class of homoplasy, convergence, is superficial similarity in morphological structures in which the underlying ontogenetic mechanisms are distinct. I argue that instances of convergence and parallelism are more common than is generally realized. Convergence suggests flexibility in underlying ontogenetic mechanisms and may be indicative of developmental processes subject to phenotypic plasticity. Parallelisms, on the other hand, may characterize developmental processes subject to constraints. Distinguishing between homology, parallelisms and convergence may clarify broader taxonomic patterns in morphological evolution. *J. Exp. Zool. (Mol. Dev. Evol.) 288:1–20, 2000.*

As the fields of developmental and evolutionary biology continue to converge, an underlying pattern is beginning to emerge: namely that the astonishing diversity of morphological variation in plants and animals is built on a scaffolding of a seemingly quite limited set of developmental programs. This pattern was predicted at least as far back as the mid-1970s by Emile Zuckerkandl ('76), at which time he wrote:

> Functional innovation at the morphological level does not appear to require any functional innovation at the molecular level. One may wonder whether the formation, in the course of evolution, of a limb of a land vertebrate from the fin of a fish requires the appearance of new proteins endowed with novel functions. I am inclined to believe that it does not. (p 404)

So when we catch our collective breath from "startling" findings such as the apparent conservation of *Hox* and *Pax* gene functions, let us step back for a moment and consider the implications. There are optimists who feel that since the set of developmental mechanisms appears to be limited, we are likely to come to an understanding of the basic nature of metazoan development and evolution via a reductionist developmental and molecular approach. I argue, on the contrary, that since innovations are manifest at the morphological level, it is necessary to integrate a morphological with a molecular approach if we hope to uncover any such underlying principles. Specifically, I feel that the study of incidents of parallel and convergent evolution may offer the best approach to accomplish this integration.

Before continuing, I now provide definitions for several key terms. "Parallelism" is independent evolution using the same mechanism. This is contrasted with "convergence": independent evolution using an alternate mechanism. The third term in this class is "reversal," or a return to an ancestral mechanism (a special case of parallelism). "Homoplasy" is a broader term covering parallelism, convergence and reversal: similarity not resulting from common ancestry. The definition of "homology" is certainly more contentious (reviewed in Donoghue, '92; Abouheif et al., '97). I tentatively adopt the definition of Van Valen ('82):

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"resemblance caused by a continuity of information." This definition highlights an important distinction between homology and homoplasy, as the latter involves a definite historical discontinuity of information. We should proceed with the realization that in practice the distinction between homology and homoplasy is often far from clear and that other definitions of homology (such as the "building block hypothesis" of Wagner, '95) may often prove more useful as heuristic devices.

The term "mechanism" in the context of convergence and parallelism is in need of some clarification. In an entirely reductionist approach, "the same mechanism" refers to identical genetic bases. For example, paedomorphosis (reproduction at an immature morphological stage) has evolved several times independently in tiger salamanders (reviewed in Shaffer and Voss, '96). Under the strict reductionist view, these independent instances of paedomorphosis are considered to have evolved in parallel only if they are characterized by mutations in the same gene or genes. Yet, discovery of the genetic bases for homoplastic changes in many organisms is not practically feasible. Thus, partly for practical reasons, I adopt a developmental definition of mechanism. If the cell biological and morphogenetic changes are identical, then I consider it to be an example of a parallelism. Furthermore, since developmental mechanisms often appear to be characterized by dense networks of cross-regulatory and feedback interactions among genes, changes in several different members of a given gene network could produce identical morphogenetic phenotypes. I consider such examples to be parallel evolution at the level of the developmental mechanism. In the case of the tiger salamanders, precocious sexual development versus delayed adult development represent different developmental mechanisms leading to a similar paedomorphic phenotype and, therefore, an example of convergent evolution at the level of the developmental mechanism. Alternatively, all tiger salamanders might be characterized by delayed adult development via blocks in the metamorphic pathway. Even if these blocks were at different points in the metamorphic pathway (which would be an example of convergence at the genetic level, since mutations in different genes would underly the instances of homoplasy), the developmental trajectories would be similarly altered and thus would represent a case of parallel evolution at the level of the developmental mechanism. Although the specific developmental mechanisms leading to paedomorphosis are poorly understood among various tiger salamanders (Shaffer and Voss, '96), when salamanders as a whole are considered, different steps along the thyroid hormone response axis are blocked in different paedomorphic species (Frieden, '81; Yaoita and Brown, '90), leading to a similar metamorphic failure. Thus although the genetic bases of the paedomorphic phenotype are clearly different (i.e., convergent at the genetic level) in different salamander species, similar developmental mechanisms (at the level of the gene network) appear to be involved, so I consider these disparate cases of paedomorphosis to have evolved in parallel. In order to learn how development may influence evolution, we should focus on similarities and differences at the level of the developmental mechanism (rather than, say, the genetic level[1]) when making evolutionary comparisons.

In Part I of this paper, I concentrate on some high profile examples in the literature where homology has been cited as the cause of similarity between arthropods and chordates. It seems that the possibility that these similarities are due to parallel or convergent evolution has been dismissed too readily. I argue that the apparent underestimation of the role of homoplasy in metazoan evolution skews our perspective on how development evolves. In Part II, I explore the possibility that the study of well-documented cases of homoplasy not only yields useful information on evolutionary patterns, but also offers significant insights into the ways in which interacting networks of regulatory genes work to produce complex morphological structures. I distinguish between cases of parallel evolution, which may indicate the presence of developmental constraints (a bias in the production of phenotypic variation due to ontogenetic factors; Maynard-Smith et al., '85), from instances of convergent evolution, which, I hypothesize, correlate with phenotypic plasticity (environmentally induced alterations in morphology within a genotype) in underlying developmental mechanisms.

PART I: HAS NOTHING INTERESTING HAPPENED SINCE THE CAMBRIAN?

With the advent of DNA sequence analysis, the word "homology" is suddenly in every molecular biologist's vocabulary. References to "the vertebrate homolog" of, say, a *Drosophila* gene are not only commonplace but, indeed, are becoming the

[1]Still, considering homplasy at the genetic level is useful in other contexts, such as understanding the evolution of protein function (see below).

cornerstone of discourse in modern developmental genetics. In addition to the inherent problems in using the same term to describe similarity at two different levels (morphological and molecular), there is a disturbing trend towards relaxing the rigor applied to the term "homology," even when conversation is restricted to sequence similarity. Thus, whatever Genbank spits out becomes a homolog of the gene being studied, with some genes being "more homologous" than others. I have no problem with the notion of degrees of homology (see Roth, '84), but as do Aboueif et al. ('97) and Doolittle ('86), I strenuously object to the equation of similarity with homology, for it leaves out the essential historical component of the term. The result appears to be that the concept of homoplasy has been trampled underfoot on the yellow brick road to a unifying principle of development.[2]

The fact that developmental networks (such as *Notch/Delta*, steroid hormones and their receptors; *Pax6/eyes absent; patched/hedgehog*) are utilized in a multitude of tissues within a single organism points to their essentially modular nature. The analogy here is to the concept of serial homology. I believe that herein lies the basis of much of the confusion in the literature of late regarding "process homology" (see Gilbert et al., '96). If a developmental module can be redeployed within an organism in the development of a variety of different tissues, then we should use extreme caution in assigning the term "homology" to the discovery of the use of the same module in two different organisms. The latter discovery suggests that the module was present in the common ancestor, but it does not show, for example, that the morphogenetic process in which it is used was also present in that common ancestor and regulated by the module in question (see also Abouheif et al., '97).

It could be argued that it all started with the *Hox* genes. The discovery that both flies and mice not only have similar *Hox* gene clusters, but also that they are ordered in the genome and expressed in the embryo in a similar manner, sent shockwaves through the developmental biology community. What followed was a flurry of conclusions (not hypotheses or suggested experimental tests) that the deuterostome-protostome ancestor had the full complement of *Hox* genes expressed in a nested anterior-posterior progression. Data on three more phyla (for a total of 5 out of about 30) led Slack and colleagues ('93) to suggest that this conserved expression of *Hox* genes is the "zootype": the primary synapomorphy (shared, derived feature) uniting the metazoa! Recently, De Robertis ('97) stated that the similar expression patterns of the *engrailed* gene in insects and cephalochordates "*tells us* that segmentation was present in the common ancestor from which the insect and chordate lineages diverged 500 million years ago" (my emphasis). There is no indication that these authors ever considered the possibility that these similarities might be due to parallel evolution.

Hox *genes and primary axis specification*

What is the appropriate test of the hypothesis that nested expression of *Hox* genes defines the metazoan body plan? The *Hox* gene cluster should be present in every metazoan, and the genes should be expressed during development in a nested orientation along the primary axis. Since 1993, *Hox* gene expression patterns have been examined in at least three other phyla: Cnidaria, Echinodermata and Urochordata (Fig. 1). The expression pattern of the only *Hox* gene (an apparent group 1/labial ortholog) that has been examined in ascidians (a urochordate) is similar to that of other chordates (Katsuyama et al., '95). *Hox* genes are apparently not expressed at all during primary axis formation in the cnidarians that have been examined, much less in nested sets (P. Cartwright, personal communication). As for echinoderms, only two of the ten identified *Hox* genes in the sea urchin *Strongylocentrotus purpuratus* are expressed during embryonic development (Arenas-Mena et al., '98), and the functions of neither are consistent with a role in positional identity along the primary axis (Ishii et al., '99). *Hox* genes are expressed at metamorphosis in cnidarians and echinoderms, but it is not clear if the genes are involved in axial patterning at these later stages. In either case, these results appear to contradict the concept of the zootype. Still, it is possible that the protostome-deuterostome ancestor had an embryonic, nested expression of *Hox* genes and that this pattern was lost in the highly derived echinoderm lineage. Clearly further studies with additional phyla are warranted (Fig. 1). Recent evidence suggesting that acoel flatworms occupy

[2]A particularly cogent example comes from a paper by Haerry and Gehring ('97, p 12) in which they speculate that "*Hoxa-4* may represent a closer relative to the *Drosophila Dfd* gene than *Hoxb-4*, since the *Hoxa-4* intron is functional in *Drosophila*, whereas that of *Hoxb-4* is not." This is essentially the same fallacious argument used to ascribe to *sonic hedgehog* the status of closest relative of *Drosophila hedgehog* on the basis of both being involved in appendage development. The argument becomes circular when it is then concluded that insect and vertebrate limbs are homologous. I discuss two further examples (*tinman/Nkx2-5* and *sine oculis/Six3*) below.

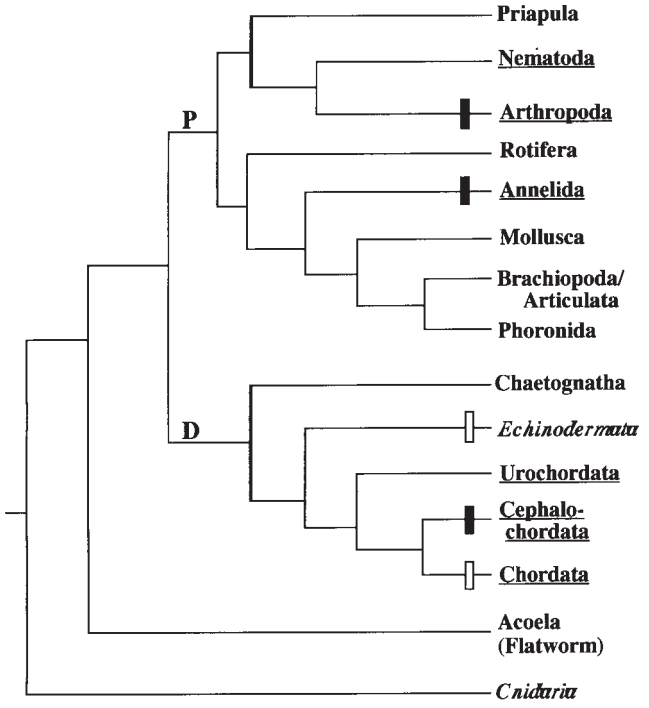

Fig. 1. Metazoan phylogeny (after Ruppert and Barnes, '94; Philippe et al., '94; Halanych et al., '95; Aguinaldo et al., '97; Ruiz-Trillo et al., '99). Here I have represented a hodge-podge of these various phylogenetic hypotheses. Different topologies yield similar conclusions. Underlined phyla have *Hox* genes expressed colinearly during primary axis formation, while the italicized phyla do not (see the text). In the other taxa (neither underlined nor italicized), *Hox* gene expression patterns have not been reported. Examination of *Hox* gene expression in phyla such as Chaetognatha, Rotifera, Priapulida, and Acoela would more fully test the "zootype" hypothesis (Slack et al., '93). Solid bars denote phyla showing a metameric pattern of *engrailed* expression. Open bars denote phyla that do not show such a metameric *engrailed* pattern. The echinoderms are represented by an open bar since the presumed ancestral condition is nonmetameric expression of *engrailed* (see Fig. 2). Although chitons (Mollusca) show a metameric *engrailed* expression pattern (Jacobs et al., '94; Jacobs, personal communication), I consider the ancestral state to be equivocal for molluscs, in which metamerism is generally believed to be a derived condition (see the text). "P" and "D" denote the protostome and deuterostome clades, respectively.

a phylogenetic position near the protostome-deuterostome divergence (Ruiz-Trillo et al., '99) make them an especially useful taxa for examining such issues.

Still, from the available data some interesting patterns are apparent. For example, the nested expression of at least the posterior class of *Hox* genes may have been independently derived in the arthropod lineage. Averof and Akam ('95) found that the anterior borders of the *Antp*, *Ubx*, and *abd-A* expression domains all coincided in the first thoracic segment of *Artemia*. In more derived arthropods (including insects and other crustaceans), the expression of these posterior *Hox* genes is nested, with the canonical, nonoverlapping anterior borders (Averof and Akam, '95; Averof and Patel, '97). While the ancestral state remains equivocal until more basal arthropod species are examined, the intriguing possibility is raised that there may be something about the organization of *Hox* genes in a cluster that predisposes them to be expressed in a nested anterior-posterior progression with respect to their chromosomal position (known as "colinearity").[3] Put simply, nested *Hox* gene expression may be a character subject to parallel evolution, not only within the Arthropoda, but indeed among the "higher" metazoa as a whole. This hypothesis is eminently testable: we simply need to examine more species. Recently, Abzhanov and Kauffman ('99) have examined the patterns of expression of the anterior class *Hox* genes in the developing head of the isopod crustacean *Porcellio scaber*, and compared these patterns to the previously reported insect *Hox* gene expression patterns. Surprisingly, while colinearity of the three head *Hox* genes examined was still observed (as it is in insects), different *Hox* genes were expressed in homologous segments. For example, while in insects, the maxillary and labial mouth parts express the *Hox* gene *proboscipedia*, the homologous appendages in the isopod were found to express *Sex combs reduced* instead. The authors concluded that arthropod head segmentation must have evolved prior to the segmental expression of the anterior class *Hox* genes. In this scenario, colinearity of anterior class *Hox* gene expression in crustaceans and insects evolved in parallel.

Furthermore, while colinearity of the anterior borders of *Hox* gene expression is a fairly consistent pattern, the *functions* of these *Hox* genes do not always correspond to this pattern. For example, while the anterior borders of *Hox*a7, *Hoxb6*, *Hoxb7*, and *Hoxb9* expression in the mouse are at different axial positions, mutations in each of these genes result in defects in the same vertebrae (Chen et al., '98). As these authors note, simi-

[3]There are two known counter-examples to colinearity. The *C. elegans ceh-13 Hox* gene is out of order with respect to chromosomal position (reviewed in Bürglin and Ruvkun, '93), but is still expressed and functions in the anterior of the animal (Brunschwig et al., '99), as is expected from its sequence similarity to other anterior-class *Hox* genes. The *Hox-B1* gene in mice is a bona fide class 1 *Hox* gene and is ordered on the chromosome in the expected position, yet its anterior border of expression lies posterior relative to that of *Hox-A2* and *-B2* (reviewed in Krumlauf, '93).

lar results have been obtained from other *Hox* gene knockouts. Again, the possibility arises that *Hox* genes are predisposed to be expressed colinearly, despite the fact that they do not always function colinearly.

These studies on *Hox* gene expression patterns in metazoan embryos exemplify some shortcomings in the field of comparative developmental biology. For example, we currently have no satisfactory biochemical model to explain colinearity. Until we understand the mechanistic basis for colinearity, it seems premature to conclude that colinearity in arthropod and chordate embryos is due to shared history. The scenarios outlined above for the possible parallel evolution of posterior and anterior class *Hox* gene colinearity in arthropods represents another possibility. A second commonly encountered problem in comparative studies is the assignment of molecular orthologies to related genes in different phyla (I discuss several additional examples below). With the *Hox* genes in particular, it is often difficult to assign relatedness among members of the vertebrate *Hox* cluster and their counterparts in other phyla. Reports of gene cloning in development and cell biology journals rarely include alignment comparisons beyond the most similar gene and often utilize only the default alignment settings in the one alignment program of choice. Furthermore, different alignment programs and different alignment parameters can yield trees with very different topologies. Thus it is extremely difficult to judge the basis on which authors claim that such-and-such a fly gene is the "closest homolog" of a mouse gene, claims that subsequently propagate through the literature (see footnote 2). Relatedness among different *Hox* genes is generally assigned on the basis of similarity within the highly conserved "homeodomain," the region responsible for DNA binding. Consensus sequences for the assignment of *Hox* genes to so-called "paralog groups" (related genes that can be traced back to gene duplication events) are based on just a few nucleotides in the homeodomain and adjacent regions (Sharkey et al., '97). Needless to say, the greater the time since two *Hox* genes diverged, the more difficult it is to assess relatedness. Finally, Avise points out ('94, p 12–13) that clusters of related genes are subject to concerted evolution (unequal crossing over leading to greater sequence similarity between neighboring genes than one would predict based on the time since gene duplication). Thus, orthologous genes (which, by definition, diverged more recently, at around the time the species being compared diverged from one another) can end up being less similar than genes within a cluster (which formed long before the divergence of the species in question). In such instances, of which the *Hox* gene cluster may represent such an example, assigning "homology" to pairs of genes in different phyla is at best a questionable exercise.

Engrailed and segmentation: homology or homoplasy?

Of the three phyla from which we have significant functional data on developmental genes (nematodes, arthropods, and chordates), two of them are characterized by overt segmentation. Segmentation in the strictest sense ("a precise and definite repetition of all [mesodermal] structures in each [body region]"; Willmer, '90, p 40) probably only applies to annelids, arthropods and chordates, while metamerism (serial repetition of some body structures) is more widespread in the Metazoa. It is found, for example, in turbellarian flatworm guts, nemertine gonads, rotifers, strobilizing cnidarian medusae, much of the Vendian fauna, brittle star and chiton body plates, and tripartite lophophorates, chaetognaths and pterobranchs. According to the suggestion of De Robertis (see above and '97), these disparate cases of metamerism might all (with the exception of cnidarians and the Vendian fauna) be variations on a segmented body plan already present in the protostome-deuterostome ancestor. Therefore, segmentation in chordates and arthropods are considered homologous. This implies that segmentation (indeed any trace of metamerism) has been lost numerous times in the evolution of "higher" metazoans but has been retained in the chordate and arthropod lineages. Again, the evidence presented for this "ancient segmentation" view is that the *engrailed* gene in annelids, arthropods and lower chordates is expressed in segmentally-iterated stripes (Holland et al., '97; Fig. 1).

These data suggest a hypothesis that segmentation is the ancestral condition (ancient segmentation) and a null hypothesis of parallel evolution. So how do we go about testing this hypothesis? Without any paleontological data, this hypothesis is very difficult to test directly. I propose that we look to the more widespread occurrence of metamerism among metazoans as an evolutionary model. Are independently evolved metameric structures built using similar (parallel) or different (convergent) developmental pathways? To answer this question one should: (1) establish via an independent phylogeny that metamerism in the

group of interest is likely to be a derived condition; (2) examine the developmental expression of *engrailed* in the group of interest, as well as in a non-metameric outgroup; and (3) compare these results to *engrailed* expression patterns in cephalochordate and insect embryos. Absence of a striped *engrailed* expression pattern in the group of interest tells you very little, but a striped pattern suggests that *engrailed* is a character subject to parallel recruitment in a similar process (metameric organization), thus casting doubt on the ancient segmentation hypothesis.

Let us examine the occurrence of metamerism in the arm plates of brittle stars, which is almost certainly a derived condition within the echinoderms (from phylogenetic as well as from paleontological data; Ruppert and Barnes, '94). Although there is no striped pattern of *engrailed* during development in sand dollars and starfish (C. Lowe, personal communication), brittle stars show an obvious striped pattern of *engrailed* expression during arm growth (Lowe and Wray, '97; Fig. 2). These results suggest that a striped pattern of *engrailed* may have evolved independently within echinoderms in the brittle stars, suggesting that expression of *engrailed* in stripes is not always evidence of shared history (though this conclusion would be bolstered if *engrailed* in crinoids is shown not to be expressed in stripes; see Fig. 2). Similar cephalochordate and arthropod *engrailed* patterns might also be due to parallel evolution. Furthermore, Chiton embryos show a striped *engrailed* expression pattern (Jacobs et al., '94, personal communication), and metamerism in chitons is generally believed to be a derived condition (Kozloff, '90; Willmer, '90; Ruppert and Barnes, '94).

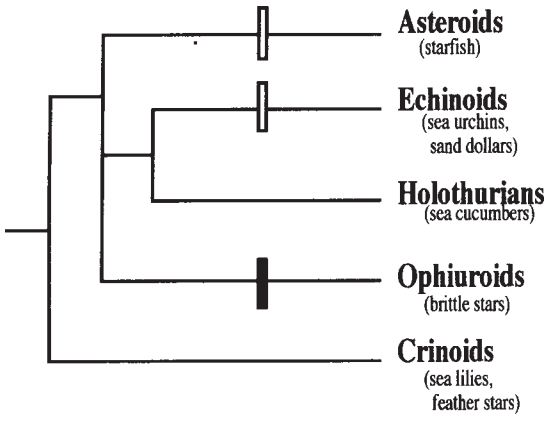

Fig. 2. Echinoderm phylogeny (Smith, '97) showing the relationships among the five extant classes. Ophiuroids are unique among the echinoderms in having strongly metameric arm skeletons (often called "vertebrae"; Ruppert and Barnes, '94). Chris Lowe (personal communication) examined *engrailed* expression in echinoderms from three of these classes and found that a metameric pattern of *engrailed* expression was present in the arms of ophiuroids (solid bar), but not in echinoids or asteroids (open bars).

Interestingly *engrailed* expression is also detected within the nervous system in brittle stars (Lowe and Wray, '97), as well as in arthropods, annelids, and chordates. This suggests a possible explanation for why one might expect parallel evolution of striped *engrailed* patterns. Generally, metameric body structures contain iterations of subsets of neurons. This is true in annelid and arthropod segments (Snodgrass, '38) as well as brittle star arms (Lowe and Wray, '97). So, if *engrailed* was expressed in these nervous systems ancestrally, one would expect to then find a metameric organization of *engrailed* in derived, metameric nervous systems. This would put *engrailed* in the right place (in a segmentally iterated pattern) at the right time (during the evolutionary elaboration of segmentally repeated mesodermal structures) to be co-opted for use in the specification of other, associated metameric structures (such as mesodermal tissues). In this view, the ancestral condition is *engrailed* expression in nervous systems, and later, parallel cooption of *engrailed* for specification of metameric structures. Again, this hypothesis is testable by examining the expression of *engrailed* in additional metameric and non-metameric organisms.

In summary, I concur with Abouheif et al. ('97) that without placing evolutionary-developmental comparisons in an explicitly phylogenetic context, it is impossible to distinguish between homology and homoplasy. Furthermore, the general assumption that such similarities are due to homology is creating a bias in research programs in this relatively young field.

PART II: PARALLEL (AND CONVERGENT) NETWORKS

Pax6 *in protostomes and deuterostomes: parallelism or homology?*

One of the classic examples of convergence in the metazoa is the evolution of the complex eye. Because the ultrastructure and developmental trajectories of invertebrate and vertebrate eyes are so different, the independent evolution of these structures had been widely accepted. Therefore, it was surprising to find that the *Pax6* gene is a key regulator of eye development in both mice and fruit flies (Quiring et al., '94). These results led Quiring et al.

('94) and others to propose that the vertebrate and invertebrate eyes are, indeed, homologous. Examination of the expression patterns of *Pax6* in a wide variety of invertebrate embryos showed that *Pax6* expression always correlated with eye development (reviewed in Callaerts et al., '97).

This example really brings into focus the problems encountered with the use of the word "homology" to describe both molecules and morphology. Yes, the "homologous gene" (properly, the "orthologous gene") is used to build both the fly and the mouse eye. But are they used in the same way? The appropriate way to address this question is *not* to see if the mouse gene works in fly eye development. The mouse gene was found to regulate eye development in *Drosophila* (Halder et al., '95), yet there is a functional *Pax6* in *C. elegans*, an organism that lacks eyes altogether (see below). Based upon its sequence similarity, I wager that *C. elegans Pax6* would also work in fly eyes. A positive result tells you only that the biochemical properties of the protein have been conserved, not necessarily that its function within a certain morphological structure has also been conserved. The commonplace use of the same gene within an organism performing distinct functions in a multitude of tissues reveals why this experiment is generally uninformative with respect to evolutionary history (see also Abouheif et al., '97). Instead, one way to address the potential similarity in function of fly and mouse *Pax6* is to examine the black box in between transcription factor and morphological structure.

There are two ways to account for the similarity in *Pax6* expression in invertebrate and vertebrate eyes. In the first scenario (which I call the "ancient eye" scenario), the protostome-deuterostome ancestor had an eye of sorts under the control of *Pax6* (as, for example, a regulator of a light-sensitive, proto-rhodopsin gene), and this association continued and was reinforced as the respective eyes increased in complexity. In the second scenario (which I call the "parallel recruitment" scenario), the *Pax6* regulatory network was independently recruited to function in invertebrate and vertebrate eye development. If the only described function of *Pax6* was in eye development, then the ancient eye scenario seems fairly likely. This is not the case: *Pax6* is also involved in brain, nose, and pancreas development in mice (reviewed in Dahl et al., '97) and head and sensory neuron development in *C. elegans* (Chisholm and Horvitz, '95; Zhang and Emmons, '95). Different *Pax6* alleles in flies show embryonic, larval or pupal lethality, head defects, and supernumerary antennae in addition to eye defects (Shatoury, '63; reviewed in Harris, '97). In fact, if metazoan *Pax6* expression patterns are considered as a whole, involvement in the development of anterior structures is probably as accurate a characterization of similarity as involvement in eye development.

In 1994, Quiring and colleagues proposed that *Pax6* may be the master regulator of eye development in mice and flies, and perhaps in all metazoans. The justification for bestowing this title upon *Pax6* was the finding that ectopic expression of the mouse or fly *Pax6* gene in, for example, the fly leg primordium results in an ectopic eye on the adult leg. It seems to me that the concept that there is a master regulator of eye development at all predisposes us to think of metazoan eye development as representing one conserved developmental program. First, homozygous null mutations in mouse *Pax6* do not block formation of the optic cup, suggesting that there is something that acts earlier than *Pax6* in mouse eye development. Furthermore, ectopic expression of *Pax6* is not the only way to produce ectopic eyes in flies. Ectopic expression of *eyes absent* (Bonini et al., '97) and *dachshund* (Shen and Mardon, '97) also have this effect (and like good members of a gene network, *dachshund* and *eyes absent* have also been shown to regulate *Pax6* expression, and vice-versa: Halder et al., '95; Bonini et al., '97; Shen and Mardon, '97). Also, a mouse *sine oculis* family member, *Six3*, can induce ectopic eyes in fish (Oliver et al., '96). So, if *Pax6* is not the master regulator of eye development, what does it do? It does regulate vertebrate lens crystallin gene expression (reviewed in Cvekl and Piatigorsky, '96). Also, Sheng et al. ('97) showed that *Pax6* directly regulates *rhodopsin 1* expression in *Drosophila* photoreceptor cells and that the promoter elements that bind *Pax6* are also found in some vertebrate *opsin* regulatory regions. This suggests a plausible scenario for the function of the *Pax6* regulatory network in eye development. The *Pax6*/*dachshund*/*eyes-absent*/*sine oculis* regulatory network may have been involved originally in specification of various anterior structures in primitive metazoans, and since it was in the right place, it was recruited to regulate the expression of structural genes in the eye (such as lens crystallins, and perhaps opsins) in invertebrates and vertebrates independently ("right place at the right time" hypothesis; see below). Uncovering the nature of this regulatory network, as well as its downstream targets, should allow us to distin-

guish between the parallel recruitment and ancient eye scenarios.

Recent data on the functions of *sine oculis* family members may support the parallel recruitment scenario. Oliver and colleagues ('95a,b) found three mouse genes with close sequence similarity to *sine oculis*, which they named *Six1*, *2*, and *3*. *Six3* is expressed in the developing eye and other anterior structures, while *Six1* and 2 are expressed in other anterior structures but not in the developing eye. Of these three genes, *Six3* has the lowest percent sequence similarity with *sine oculis*, yet Oliver et al. ('95b) conclude that *Six3* is the "functional homolog" of *sine oculis* since both are expressed during eye development. As it turns out, there is another *Drosophila* gene in this family called *optix* which is also expressed during fly eye and head development. Sequence comparisons with the Six genes revealed that *optix*, not *sine oculis*, is the *Six3* ortholog in flies (Toy et al., '98). However, this still leaves us with the unsavory situation that *sine oculis* is involved in eye development while its mouse orthologs (*Six1* and *2*) are not. The question is: what was the role of *sine oculis* in the protostome-deuterostome ancestor? The most obvious answer is that, like all the members of this family, it was probably involved in anterior development and later was recruited into the development of the fly, but not the mouse, eye.

If the ancient eye scenario is correct, it tells us that when you have a system that works, you conserve it. The parallel recruitment scenario, by contrast, offers us a model for understanding how complex structures are built in multicellular organisms. If squid, flies, and mice have all come up with the same solution to the same problem independently, this suggests that metazoan eye development is "constrained" in some fashion. This could be for two (not necessarily mutually exclusive) reasons. First, if we hypothesize an eyeless metazoan in which these genes were already expressed in the head, they would be in the right place (the head) at the right time (namely, the time at which eyes evolved in the respective lineages) to be recruited for an additional role in eye development (as described above). Alternatively, there may be something about the biochemical nature of the *Pax6* network that makes it particularly good at regulating the inductive interactions characteristic of both vertebrate and invertebrate eye development. Once again, our understanding of the biochemistry of this system is far too rudimentary to be able to evaluate this possibility. Developmental constraint in this context refers to the predisposition of the developmental system to utilize the *Pax6* network in eye evolution. Put another way, constraint in developmental programs (such as the *Pax6* network) may be especially likely to lead to parallelisms in morphological evolution.

Developmental constraints and parallel evolution

I believe that the clearest definition of developmental constraints was proposed by Maynard Smith et al. ('85) as a bias in the production of phenotypic variation due to ontogenetic factors. Wagner and Misof ('93) noted that "constraint" can make itself evident at the level of the phenotypic character (morphostatic constraints) or the underlying developmental processes (generative constraints). Morphostatic constraints generally imply variability in generative processes and vice-versa. Von Dassow and Munro ('99) provide a useful example. Different insects utilize distinct generative processes to specify segmental identity during embryogenesis. In the short-germ insects, typified by *Drosophila melanogaster*, all the segments are specified nearly simultaneously in a noncellular, syncitial environment (reviewed in Lawrence, '92). By contrast, in the long-germ insects, typified by the grasshopper *Schistocerca*, the segments are specified in a gradual anterior-to-posterior progression in a cellular environment (reviewed in Patel, '94; Tautz et al., '94). Still, both processes eventually generate very similar-looking segmented embryos, with the same numbers of head, thoracic and abdominal segments. In Wagner and Misof's terminology, substantial variation in developmental (generative) processes underlie the production of a constrained segmental (morphostatic) phenotype.

On the other hand, variation in the axial position and morphology of insect appendages (morphostatic variability, such as the number of wings and wing-like structures in different insect groups) is associated with tight conservation of *Hox* gene expression patterns (a possible generative constraint). I believe that the explanation for these findings is that constraint at each level underlies the essential modularity of development. In the case of morphostatic constraints, a phenotypic character is maintained despite variations in the trajectories of development, such as heterochronies or canalization in the face of environmental heterogeneity and the production of segmented insect embryos. In the case of generative con-

straints, conserved developmental pathways are utilized for the production of different phenotypic characters, such as *Pax6* function in the vertebrate pancreas and the fly eye and appendage variability in insects.

Although developmental constraints are often invoked to explain biases in the patterns of morphological variation, in practice it is far from straightforward to determine if the lack of variability in a morphological feature is due to constraints or, for example, stabilizing selection on that morphology (see Maynard-Smith et al., '85). The presumed explanation for this difficulty is the general lack of knowledge about the mechanistic bases for supposed constraints. Recently, however, some work on trade-offs (van Noordwijk and de Jong, '86; Houle, '91; de Jong and van Noordwijk, '92) in insect development has shed some light on a potential mechanism for constraints. Nijhout and Emlen ('98) and Klingenberg and Nijhout ('98) noticed trade-offs in the growth of morphological features in horned scarab beetles and buckeye butterflies. The beetles have two distinct male morphotypes: larger beetles have long horns and small eyes while smaller beetles have short horns and large eyes. Selection for beetle lines with long or short horns always yielded beetles with, respectively, decreased and increased eye size. Furthermore, the application of juvenile hormone (JH) during larval development (which is known to control the difference between the two morphotypes) results in short-horned beetles developing a larger body size. These beetles also develop large eyes, while females (which never produce horns) show no significant change in eye size with JH treatment. In these experiments, the sizes of other body parts were not affected. It seems that eye size and horn size are truly (and negatively) coupled. Experiments with the buckeye butterfly showed that removal of the hindwing primordium in a caterpillar resulted in an adult with significantly increased weights of the forewing, thorax, and foreleg. Again, other body parts were unaffected. Interestingly, the increased forewing and foreleg weights were only apparent on the same side of the animal as the hindwing removal: the forewing and foreleg on the opposite side of the animal were essentially unaffected (controls demonstrated that this was not simply due to surgery). These findings led the authors to conclude that there is competition for a local resource in these insect larvae, a form of internal trade-offs. Alterations in the size of one body part appear to be constrained by correlated alterations in the size of nearby body parts. Such constraints apparently influence evolvability (evolutionary potential; see below) since horn size is quite variable among related horned beetle species, but is always negatively correlated with eye size (D. Emlen, personal communication). Wagner et al. ('97) also discuss an example of mechanisms of constraints with respect to digit formation in salamanders (see also Wilkins, '98; von Dassow and Munro, '99).

The "right place at the right time" hypothesis, heart evolution, and developmental constraints

Although morphologically quite distinct, development of both the vertebrate and fly heart is regulated by members of the *NK-2* family of genes: *tinman* in flies and *Nkx2–5* (among others) in mice. This finding has led to the now popular notion that the protostome-deuterostome ancestor had a *NK-2*–regulated heart, which contrasts with the traditional view of convergence between these two organs (e.g., Beklemishev, '69). *Nkx2–5* and *tinman* are first expressed broadly in the visceral mesoderm and only later restricted to cardiac mesoderm. Ranganayakulu and colleagues ('98) set out to test whether the roles of *NK-2* genes in flies and mice are due to homology or to parallel evolution by substituting the mouse gene for the fly gene in transgenic flies. Surprisingly, while the mouse gene rescued the visceral mesoderm defects found in *tinman* mutants, it failed to rescue the heart defects! Furthermore, they mapped the domain of the *tinman* protein responsible for the heart function to a small region at the N-terminus, a region of the protein that shows no detectable amino acid similarity with *Nkx2–5*. These findings suggest that while one of the ancestral roles of *NK-2* genes in the protostome-deuterostome ancestor may have been in visceral mesoderm development, *Nkx2–5* and *tinman* appear to have been independently co-opted for heart development in the two lineages, an example of parallel evolution.[4] Further evidence to support this idea comes from sequence comparisons of *Nkx2–5* and *tinman*. Here, again, as was the case for the *Six* genes discussed above, we find a situation in which the true orthologs (the genes showing the highest sequence similarity to one another) do not appear to share the same role. All the *NK-2* genes

[4]Of course, the formal possibility exists that the control sequences for heart within the two proteins have diverged enough (from a common ancestor's control sequence) to make it unrecognizable not only by DNA alignment methodology, but also by the functional analysis described above.

involved in vertebrate heart development are actually closer relatives of the fly *ventral nervous system defective* and *bagpipe* genes (Ranganayakulu et al., '98), which function in the nervous system and visceral mesoderm, respectively.

In this parallel recruitment scenario for heart evolution, the "developmental constraint" is an example of the *NK-2* genes being expressed in the "right place at the right time" (i.e., in the visceral mesoderm during the time that hearts evolved) in both the protostome (e.g., insect) and deuterostome (e.g., vertebrate) lineages. Metazoan hearts arise from visceral mesoderm, and *NK-2* family genes were probably involved ancestrally in the determination of these tissues. This should be considered a bona fide constraint on heart evolution, since it certainly represents a "bias [in] the production of variant phenotypes caused by the…dynamics of the developmental system" (Maynard-Smith et al., '85, p 266). Furthermore, the reiterated use of a gene first in, say, a field of cells, and later in only a subset of the progeny of those cells is commonplace in animal development [e.g., *Distal-less* in proximo-distal axis specification in insect appendages early, and later in the differentiation of distal pattern elements in the butterfly wing (Carroll et al., '94; see below); the role of the *C. elegans Hox* gene *mab-5* in several distinct steps (with distinct functions) in male tail morphogenesis (Salser and Kenyon, '96)].

A biochemical analog of the "right place at the right time" scenario appears to hold for nuclear hormones receptors and their ligands. It appears that in at least two instances, distantly related members of the nuclear hormone receptor superfamily have acquired the ability to bind chemically similar ligands. The insect ecdysone receptor and its relatives bind steroids (Kliewer et al., '99), but are in a different sub-clade than the vertebrate steroid receptors; the vertebrate retinoid-X receptor and retinoic acid receptor are distant relatives, but both bind 9-cis-retinoic acid (Escriva et al., '97; Laudet, '97). These might be examples of "right ligand binding region configuration at the right time" and are probably examples of parallel evolution at the level of three-dimensional protein structure (see also, e.g., Govindarajan and Goldstein, '96; Graumann and Marahiel, '96). This phenomenon, whereby unrelated or distantly related genes have evolved similar biochemical activity independently, has recently been referred to as "non-orthologous gene displacement," and was shown to be quite common in a comparison of the proteins encoded by the fully sequenced genomes of two species of bacteria, *Haemophilius influe*nzae and *Mycoplasma genitalium* (Koonin et al., '96).[5] For example, RNase H activity is conferred by a typical RNase H ortholog in *H. influenzae*, while an unrelated protein with similarity to the 5´-3´ exonuclease domain of DNA polymerase I performs this role in *M. genitalium*.

I believe the *tinman* story is only the tip of the iceberg. Examples of parallel evolution are commonplace, at least among the limited instances in which people have explicitly looked for them. Although there is substantial precedence for the concept that the same genes and proteins are utilized repeatedly in morphological evolution (Jacob, '77), it is possible that current evolutionary theory, with its emphasis on parsimony, may not be well equipped to deal with the numerous findings of parallellisms (and convergence) in morphological evolution. Here I cite some of the examples that I have come across in my search of the literature, as well as an example from my own work on ovarian development in insects. I discuss these examples because in each case, the finding of parallel evolution may indicate constraints on morphological evolution. Identifying the nature of these constraints may offer insights into the evolutionary potential ("evolvability") of these lineages (see below).

Morphogenetic hormones and developmental constraints

Mangroves are a polyphyletic assemblage, with the mangrove phenotype having evolved independently in 16 families (Tomlinson, '86; Juncosa and Tomlinson, '88; Chase et al., '93), six of which show vivipary (Tomlinson, '86). Farnsworth and Farrant ('98) performed an outgroup comparison of mangroves and non-mangroves in four of the six viviparous families, monitoring the relationship between abscisic acid (ABA) levels and the evolution of vivipary and the corresponding loss of seed dormancy. ABA has been shown to be involved in the desiccation tolerance of dormant seeds as well as in plant responses to flooding and high salinity (reviewed in Kermode, '95). While one non-viviparous mangrove (*Sonneratia alba*) showed levels of embryonic ABA more akin to its non-mangrove sister group, each of the independently evolved viviparous mangrove species showed re-

[5]Eleven such cases were found, as compared to the 233 protein pairs shown to be orthologous between the two species. Extrapolating, the authors predict that such cases in animal genomes should number in the hundreds. Cases of both parallelisms and convergence are grouped in this analysis.

duced levels of embryonic ABA. Therefore, these four independent cases of evolution of viviparous mangroves appear to include a common mechanistic basis, as parallel hormonal modifications in the embryo have evolved in concert with the evolution of vivipary.

In insects, a unique type of vivipary is found in some species of gall midges (dipterans from the family Cecidomyiidae), which have evolved facultative larval reproduction (called "paedogenesis") at least two times independently (Jaschof, '98; Hodin and Riddiford, submitted). In both taxa, paedogenesis involves the precocious differentiation of the ovary in early-stage larvae (reviewed in Went, '79). The resulting oocytes undergo parthenogenetic activation, and the embryos develop in the hemocoel (open body cavity) of the mother larva. Ultimately the larvae hatch, consume the mother from the inside and burst out of the empty cuticle. The newly emerged larvae repeat the paedogenetic life cycle if resources are abundant, but delay ovarian differentiation and metamorphose into a winged adult under poor food conditions. In two of the species with independently evolved paedogenesis (*Heteropeza pygmaea* and *Mycophila speyeri*), precocious ovarian differentiation is associated with up-regulation of the Ecdysone Receptor (EcR) and Ultraspiracle (USP) proteins within 12 hours after larval birth (Hodin and Riddiford, submitted). EcR and USP heterodimerize to form the functional receptor for 20-hydroxyecdysone (Yao et al., '93), which is critical for molting and metamorphosis in insects. In *Drosophila melanogaster*, the ovary lacks both EcR and USP during most of larval life, then early in the final instar both appear and ovarian differentiation begins (Hodin and Riddiford, '98). Similarly, in both paedogenetic gall midge species, larvae that are destined to metamorphose (poor food conditions) express only low levels of EcR and USP in the ovarian primordium until metamorphosis. Thus the ontogenetic mechanisms underlying paedogenesis (precocious activation of EcR and USP) seem to have evolved in parallel within the gall midges. Since ecdysteroids appear at every molt in insects, heterochronies in tissue differentiation in insects may commonly be accomplished by changes in the timing of EcR/USP expression. In other words, the use of the ecdysteroid-receptor system to evolve heterochronic changes may represent a local evolutionary optimum (Sewall Wright, '32): in this case, a simple developmental switch may cause a macroevolutionary change in morphology and life history. It is the modular nature of adult organ formation in metamorphosing insects that would make this scenario possible.

The previous two examples, viviparous mangroves and paedogenetic insects, have major evolutionary alterations in life history patterns in common that involve modifications in hormonal systems. The evolution of neoteny in salamanders (Frieden, '81; Yaoita and Brown, '90) and direct development in frogs (Hanken et al., '97; Jennings and Hanken, '98) and possibly sea urchins (Saito et al., '98) similarly involve hormonal modifications. Perhaps this is not surprising. Since life history transformations in a wide variety of multicellular organisms tend to be regulated by hormones, modifications in hormone release and/or the cellular response to hormones are a likely focus for evolutionary change in these systems (see also Matsuda, '82; Nijhout, '99). Furthermore, I believe that these would appropriately be considered "constraints" on life history evolution, as evolutionary patterns are certainly biased in these instances.

Close ecological associations and parallel evolution

Close interactions between organisms can often lead to reiterated evolutionary patterns and may hence indicate constraints. Becerra ('97) and Becerra and Venable ('99) investigated the chemical bases of the shifts in host plant use by flea beetles (genus *Blepharida*) on their host plants, New World frankincense and myrrh (genus *Bursera*). Burseras use a variety of terpenes as chemical defenses, and distantly related burseras seem to have evolved the use of certain types of terpenes independently. Consequently, some beetle species that ancestrally appear to have exploited only one sub-clade of *Bursera* can colonize new plants that fortuitously (for the beetles) produce a similar class of terpenes. In this case, parallel evolution of terpene production within the burseras can explain macroevolutionary trends within the blepharid flea beetles. The distribution of flea beetles is to some degree constrained by the evolutionary patterns in terpene production in burseras.

Endoparasitism has evolved in hymenopteran wasps at least eight times independently from ectoparasitic ancestors (reviewed in Strand and Grbic, '97) and in each case is associated with a remarkably similar suite of developmental modifications. Early embryogenesis in hymenopterans (as exemplified by honeybees; DuPraw, '67; Fleig, '90; Fleig et al., '92; Binner and Sander, '97) generally follows the *Drosophila* pattern (reviewed in

Lawrence, '92): a large yolky egg, syncytial early nuclear divisions, cytokinesis at the "cellular blastoderm" stage, and segmental patterning via a cascade of regulatory interactions from pair rule to segment polarity to homeotic genes (maternal and gap genes have been difficult to find outside higher Diptera, but see Wolff et al., '98). Ectoparasitic hymenopterans also appear to share the canonical *Drosophila* pattern (Grbic and Strand, '98). Many endoparasites, however, have undergone radical shifts in the patterns of early development. Most endoparasites develop from tiny eggs with little or no yolk and cellularize early (Strand and Grbic, '97; M. Strand, personal communication). These parallel modifications are likely related to the fact that endoparasitic embryos take up nutrients from the fluids of the host and can therefore develop into sizable larvae from small eggs. Early cellularization might also be a function of small egg size. Most strikingly, in the two independently evolved endoparasites that have been examined for expression of patterning genes, both appear to have lost pair rule gene functions, and the earliest evidence of the segmentation cascade is the expression of segment polarity genes (Grbic and Strand, '98; Grbic et al., '98). A similar situation appears to hold in so-called "short-germ" insects (such as in grasshoppers; reviewed in Patel, '94; Tautz et al., '94), in which segments are added progressively throughout embryogenesis. Perhaps the more complex segmentation hierarchy is only required when patterning "long-germ" embryos (typified by *Drosophila*), in which all the segments are specified during a very short developmental period. Unlike some of the previous examples, the parallel alterations in early embryogenesis in endoparasites seem to indicate a relaxation of constraints on early embryogenesis in each of these lineages, leading to parallel losses of developmental processes in these insects.

Evolvability and developmental constraints

Wagner and Altenberg ('96, p 970) define evolvability (evolutionary potential) as "the genome's ability to produce adaptive variants when acted on by the genetic system." Developmental constraints can influence evolvability either by hindering the ability of a genome to produce a given adaptive variant or by predisposing genomes to produce a given variant by a defined mechanistic route (which, I argue, may likely lead to parallel evolution of this adaptive variant in related lineages). As a possible example of the latter, let us consider the evolutionary potential of bacterial β-galactosidase. Although bacteria do not undergo development per sé, a recent study by Hall and Malik ('98) offers some insights into evolvability, and thus developmental constraints. An *E. coli* gene known as "evolved β-galactosidase" (Ebg) is essentially incapable of effectively utilizing β-galactoside sugars. Extensive mutagenesis has shown that only mutations causing amino acid replacements at two of the 15 active site residues of Ebg can restore effective function. Furthermore, a phylogenetic comparison of 13 related bacterial β-galactosidase genes revealed that the β-galactosidase consensus differs from Ebg only in these same two active site residues. It seems that the evolutionary potential of Ebg to evolve into a functional β-galactosidase is limited to these two amino acid replacements. This may well be an example of a local optimum; perhaps other active conformations are possible but may involve too many intermediate evolutionary steps to make the evolutionary jump likely. Alternatively, there may simply be no possible alternate configurations. In either case, this seems to be a nice biochemical demonstration of an evolutionary constraint. An analogous situation may explain the remarkable similarity between the independently evolved antifreeze glycoproteins in Antarctic notothenoid fish and Arctic cod. Although both proteins are characterized by Thr-Ala-Ala amino acid repeats, the notothenoid gene evolved from an ancestral trypsinogen gene (Chen et al., '97a), while the cod gene is completely unrelated to trypsinogens (Chen et al., '97b). Therefore, the similar functional requirements in the two fish have led to the entirely independent evolution of Thr-Ala-Ala–containing proteins. Like the Ebg gene example, either the biochemical options for this particular functional role (antifreeze) are quite limited, or it is relatively easy (perhaps due to codon bias) to evolve Thr-Ala-Ala repeats.

Occasionally parallel evolution may simply be the result of chance. I believe that the use of aldehyde dehydrogenase as a lens crystallin in squid and elephant shrews represents such a case (Tomarev et al., '91; Wistow and Kim, '91; Graham et al., '96). There are many different metabolic enzymes utilized as lens crystallins in different taxa, but the set of possible proteins is probably somewhat limited. Presumably squid and elephant shrews just happened to come up with the same solution. Yet some cases of multiple similar transformations within a single lineage cannot be explained as chance occurrences. Skeletal changes in plethodontid salamanders (Wake, '91),

cleavage modifications in direct developing echinoids (Wray and Bely, '94), and extreme dimorphism in male fig wasps (Cook et al., '97) are a few striking examples. Possibly, each of the similarly transformed lineages was responding to similar selection pressures and either evolved different solutions to the same problem (convergence) or evolved the same solutions (parallelism). If the solutions are convergent, then developmental constraints are probably not acting on the structure in question. By contrast, if the solutions represent parallelisms, developmental constraints may be involved.

Still, developmental constraint is not the only explanation for multiple parallel transformations within a lineage. A purely adaptationist explanation is also possible: namely, the parallel transformations in question may represent the best possible solutions in the presence of similar selection pressures.

Developmental constraints in practice

So how can developmental constraints be positively identified? If different types of developmental perturbations (teratogens, environmental shifts, transgenics) tend to produce a similar set of phenotypes, developmental constraints may be involved. The development of the vertebrate skeletal axis is a nice example. To date, about 50% of the mouse *Hox* genes have been knocked out, and some patterns are beginning to emerge. Skeletal defects appear to be concentrated at certain "hot spots" (Chen and Capecchi, '97), notably the boundaries between different vertebral types (e.g., shifts in the lumbo-sacral border). In addition, segmental fusions (e.g., rib fusions) appear repeatedly. Interestingly, Russell ('56) noted very similar results for mouse embryos exposed to X-rays while undergoing organogenesis (6.5–12.5 days after fertilization). Irradiations at earlier and later stages had little or no effect on skeletogenesis, suggesting that the explanation for the similarity between X-irradiation and *Hox* mutants is not as simple as representing X-ray–induced lesions in *Hox* genes. An analogous situation occurs with *Drosophila Hox* mutations at the *bithorax* locus, in which the haltere-bearing segment (T3) is transformed to a wing-bearing segment (T2). Either temperature shock or ether application to embryos during a critical period around the time of blastoderm formation yields *bithorax* phenocopies (Capdevila and Garcia-Bellido, '74; and references therein; see also below).

My interpretation of these data is that axial patterning in both flies and mice is a process subject to developmental constraints and that these constraints are manifest by a limited subset of axial defects following diverse perturbations. The implicit prediction is that the vertebrate and arthropod lineages are each characterized by parallel variations in axial morphology. Recent data from Averof and Akam ('95) and Averof and Patel ('97) on *Hox* gene expression domains in various crustaceans suggest that the extreme variation in crustacean appendages overlays a stereotyped pattern of variations in *Hox* gene expression. How these differences fall out phylogenetically is not clear, so the jury is still out on arthropod axial parallelisms. Interestingly, a very similar scenario has unfolded during vertebrate axial evolution. For example, while the transition between the cervical and thoracic vertebrae occurs at widely divergent axial positions across vertebrates, the anterior border of the *Hox*c-6 expression domain is at the somite level corresponding to this morphological transition in all cases examined to date (frog, fish, mouse and birds; Gaunt, '94; Burke et al., '95).

Since the seemingly parallel alterations in axial evolution in both arthropods and vertebrates are mirrored in the transgenic and developmental perturbation experiments cited above, I conclude that axial evolution in these lineages is to some degree constrained by the expression patterns and functions of *Hox* genes.

Phenotypic plasticity and convergent evolution

Convergence is a widespread phenomenon in both plant (reviewed in Niklas, '97) and animal (reviewed in Moore and Wilmer, '97; Conway Morris, '98) evolution. Yet very few studies have explicitly examined the developmental mechanisms underlying the production of supposedly convergent structures. In a landmark study of the mechanisms of homoplasy in salamanders, Wake ('91) identified several cases in which superficially similar morphologies were produced by very different developmental trajectories, including the independent acquisition of vertebral joints by three distinct mechanisms and the evolution of vertebral elongation in tropical salamanders by either the addition of vertebrae (as in *Oedipina*) or by elongation of individual vertebrae (as in *Lineatriton*). Such instances of convergence within relatively closely related taxa indicate that the underlying developmental system is somewhat flexible, in contrast to the developmental constraints indicative of parallelisms. If we take this

one step further, developmental mechanisms shown to be convergent may indicate flexibility in developmental trajectories within a genotype, a phenomenon known as phenotypic plasticity (environmentally based phenotypic differences within a genotype; reviewed in Schlichting and Pigliucci, '98). Turning this argument around, I hypothesize not only that organisms exhibiting phenotypic plasticity for a trait tend to express interspecific variability (genetically fixed differences between species) for that trait, but also that the mechanisms underlying such variability are convergent. A corollary of this hypothesis is that lineages in which phenotypic plasticity is the ancestral condition are characterized by multiple instances of convergent evolution (Moore and Willmer, '97). To test these hypotheses I have searched the literature for cases in which both the mechanisms underlying phenotypic plasticity for some feature and the mechanisms underlying interspecific variability for that same feature are known. Additionally, in my own work I have investigated variability in ovariole number (an indicator of ovary size) in fruit flies and honeybees with the same issue in mind. It should be noted that in many instances, so-called convergent structures might be similar at only a very superficial level. For the term to have any relevance with respect to evolutionary patterns, use of the term should be restricted to similar structures that might have some aspect of their function in common (such that similar evolutionary forces might be expected to act on the two independently evolved structures).

Some experiments conducted almost 50 years ago by Waddington ('56) still have not been adequately accounted for by a developmental genetic model. Application of ether to fruit fly embryos results in some adults developing a *bithorax*-like phenotype (see above). After selection for animals that responded in this way, Waddington ('56) noticed that after only eight generations some flies developed the *bithorax*-like phenotype without any ether application at all! Waddington coined the term "genetic assimilation" to describe the phenomenon, and analysis of the spontaneous *bithorax*-like stock revealed that it was caused by a single dominant allele. Therefore, this mutation must have arisen fortuitously in the course of selection (otherwise it would have been detected in the parental population). In a second round of selection, a similar-looking, constitutive, *bithorax*-like mutation arose in generation 29. This second mutation was also a single dominant allele but at a completely different locus. Recently, Gibson and Hogness ('96) showed that the plastic *bithorax*-like response to ether[6] seems to result from localized loss of Ultrabithorax expression in the haltere primordium (which is normally required to repress wing formation there; see above). Since the developmental bases of the *bithorax*-like phenotype was not investigated for the constitutive lines, we cannot distinguish between parallelism and convergence in this case. Later, I discuss the possibility that genetic assimilation might represent a generic mechanism of evolutionary change. While reading the following examples, the reader should keep in mind the possibility that genetic fixation of phenotypically plastic traits might have occurred by a process akin to Waddington's genetic assimilation (West-Eberhard, '89; Schlichting and Pigliucci, '98).

Buckeye butterflies exhibit seasonal phenotypic plasticity ("seasonal polyphenism") for the color of the ventral hindwing: beige in the summer (the *linea* morph), dark reddish-brown in the fall (the *rosa* morph). The shift between the two forms is largely photoperiod dependent. When Rountree and Nijhout ('95a) removed the brains of *linea*-destined pupae, they tended to produce the *rosa* morph, but injection of 20-hydroxyecdysone (which is not produced in brainless animals) rescued the *linea* morph. When levels of ecdysteroids were measured in *rosa*- and *linea*-destined pupae, *rosa*-destined pupae had much lower levels of ecdysteroids during the "critical period" (the time when injection of 20-hydroxyecdysone into brainless pupae rescued the *linea* morph). Therefore, the developmental basis of the plastic response appears to be the suppression of ecdysteroid secretion during the critical period in *rosa*-destined pupae. There is also intra-specific variation for this plastic response, and there exists a constitutive *rosa* morph in natural populations. Surprisingly, constitutive *rosa* pupae do not show the suppression of ecdysteroids characterizing the plastic response. Instead, their ecdysteroid profiles resemble those of *linea*-destined pupae (Rountree and Nijhout, '95b). Transplantation of constitutive *rosa* wing primordia into *linea*-destined pupae resulted in the *rosa* phenotype, indicating that the constitutive *rosa* phenotype is due to some factor or factors within the wing disc itself, and is thus distinct from the mechanism that produces the plastic response (ecdysteroid levels; though the constitu-

[6]I use the term "phenotypic plasticity" broadly, to refer to any environmentally induced alteration in morphology within a genotype. Thus, while fruit flies in their natural habitats would never encounter ether, I consider the phenotypic effects of ether application to fruit fly embryos to be an example of phenotypic plasticity.

tive *rosa* phenotype may be due to alterations in the ecdysteroid response pathway specifically in the wing). Since the *rosa* morph is due to the expression of two additional ommochrome pigments in the wing (Nijhout, '97), it will be interesting to see how the expression of enzymes involved in the synthesis of these pigments is differentially regulated in the plastic and constitutive *rosa* morphs. In any case, the plastic and constitutive *rosa* morphs are produced by convergent developmental mechanisms.

Colder developmental temperatures result in increases in body size and wing size in fruit flies. The increases in wing size due to this plastic response in *Drosophila melanogaster* have been shown by several researchers to result from increases in cell size rather than cell number (Partridge et al., '94 and references therein). Partridge and colleagues ('94) found that selection at intermediate temperatures for increased wing size also causes increases in cell size. De Moed and colleagues ('97) showed that genetically fixed differences in wing size among three *D. melanogaster* populations reared in a common garden were explained by differences in cell number. Therefore, genetically fixed variability in wing size can be caused by either increases in cell size (the selection experiments of Partridge et al., '94) or increases in cell number [as de Moed et al. ('97) found in natural populations], and is thus another example where marked plasticity is correlated with convergent evolution.

Terminal height and leaf number are phenotypically plastic characters in two closely related species of *Lobelia* (Lobeliaceae), *L. cardinalis* and *L. siphilitica* (Pigliucci and Schlichting, '95; Pigliucci et al., '97). There is also substantial genotypic variability for these characters. When the growth trajectories of several genotypes of each species were examined, these authors noted that different genotypes can converge on similar terminal phenotypes by different developmental routes. For example, similar final height could be reached either by a spurt of rapid growth early in ontogeny or by sustained slower growth.

The insect ovary is a modular structure made up of ovarioles, each of which can independently mature eggs in an assembly-line fashion. Therefore, ovariole number correlates with reproductive output, and hence, presumably, fitness. Still, there may be trade-offs associated with having larger ovaries, such as decreased flight maneuverability (Berrigan, '91) or developmental production costs (Nijhout and Emlen, '98). Ovariole number varies widely within drosophilids (reviewed in Mahowald and Kambysellis, '80) and is even quite variable within the *melanogaster* species group. For example, while the mean ovariole number varies among populations of *D. melanogaster* from 16 to 23 per ovary, the most derived member of the species group (Caccone et al., '96), the island species *D. sechellia*, has only 8–9 ovarioles per ovary. Furthermore, ovariole number is determined during larval development, and *D. melanogaster* expresses marked phenotypic plasticity for ovariole number when reared at various temperatures or nutrient conditions (Savilev '28; Delpuech et al., '95; Moreteau et al., '97; Morin et al., '97). Hodin and Riddiford (submitted) examined the mechanistic basis for plasticity and interspecific variation for ovariole number in flies of the *melanogaster* species group by comparing the trajectories of ovarian growth and differentiation in the various phenotypic and genotypic contexts. We found that the mechanisms underlying the plastic decrease in ovariole number under different rearing conditions are mostly distinct from the mechanisms explaining interspecific variation in ovariole number. Thus, once again, plasticity was correlated with mechanistic convergence in this system.

Still, there are exceptions to this trend. For instance, honeybee larvae of a given genotype develop either into a queen with hundreds of ovarioles or into a worker with generally fewer than 10 ovarioles, depending on larval nutrition. Hartfelder and Steinbrück ('97) and Schmidt Capella and Hartfelder ('98) have shown that this difference is due to differential cell death in the ovarian primordia of workers in the honeybee *Apis mellifera carnica*. In addition, the workers of different geographical races of *A. mellifera* differ in mean ovariole number, with the most striking instance being workers of the Cape honeybee *A.m.capensis*, which have about twice as many ovarioles as do most other races, including their neighbor the African honeybee, *A.m.scutellata* (reviewed in Ruttner, '88). Our preliminary results suggest that the differences in ovariole numbers between the workers of these two African races is also due to differential cell death (Hodin, Crewe, Riddiford, and Allsopp, unpublished). Therefore, the mechanism of plasticity appears to be the same as that for intra-specific variation in this instance.

Another counterexample comes from work on butterfly eyespots. Many butterflies have large circular patterns on their wings known as eyespots, which are thought to play a role in predator avoid-

ance by startling potential predators. In the satyrine butterfly, *Bicyclus anynana*, there are two seasonal morphs determined by temperature: a warmer wet season morph with larger eyespots, and a cooler dry season morph with smaller eyespots. Expression of the *Distal-less* (*Dll*) gene had been previously shown to prefigure the position of the eyespots in the wing primordia of the buckeye butterfly (Carroll et al., '94), and a similar pattern of expression is seen in the wet season morph in *B. anynana* (Brakefield et al., '96). Pupal wing primordia of dry season morphs show a smaller patch of *Dll* expression prefiguring the position of their smaller eyespots. Brakefield and colleagues ('96) also examined butterfly lines selected at an intermediate temperature for constitutive expression of either large or small eyespots. The constitutively large-spotted morph showed large patches of *Dll* in the pupa, while the constitutively small-spotted morph showed small patches. Furthermore, recent work has demontrated that the size of the eyespots on the ventral wings is regulated by ecdysteroids, and that differences in the timing of ecdysteroid release in the large and small eye spot selection lines mirror alterations in ecdysteroid release of unslected lines reared at warmer and cooler temperatures, respectively (Koch et al., '96; Brakefield et al., '98). Thus, the mechanism of plasticity (early ecdysteroid release yielding large ventral eyespots, apparently via large *Dll* patches; later ecdysteroid release yielding small ventral eyespots, apparently via small *Dll* patches) seems to be the same as the mechanism of fixed, genetic differences in the selection lines.

It should be noted that in the *Dll* example, as well as in the example of the honeybees, there still may be instances of convergence within these taxonomic groups. Recall that on its own, Partridge et al. ('94) suggested that the mechanisms of plastic and genetically fixed differences in wing size were the same. It was only after de Moed et al. ('97) examined three additional *D. melanogaster* populations that convergence was apparent. Perhaps if more independent cases of increased or decreased ovariole number in bees, as well as interspecific variation in eyespot size were examined, some instances of convergence would be found.

Still, in six cases in which (to my knowledge) plasticity and genetically fixed mechanisms of variation have been compared, convergent mechanisms were apparent in four of them. The significance of this finding is in the notion of evolvability, and I return to the concept of modularity to exemplify this point. Phenotypic plasticity is a special case of modularity, where, for example, photoperiod shifts can produce radical changes in wing coloration while the development of many other morphological features remains unaffected. Evolutionary changes in such developmental systems are also characterized by flexibility, as evidenced by the preponderance of convergence in such cases. Parallelisms, on the other hand, are characteristic of developmental mechanisms that are more constrained, such as the relationship between *Hox* gene expression and appendage type in arthropods (Averof and Patel, '97). In such situations constraints may limit the scope of possible variation. Yet the arthropod segmental plan is clearly a successful one, so the constraint does not appear to be much of a hindrance. The "success" of constrained developmental programs may be attributed to one of two phenomena. First, the developmental system may be primed to respond to selection in a given way, thus making the evolutionary options more limited but the process of adaptation more efficient (a quicker route to an adaptive peak, in Wright's terminology). Alternatively, constraints may necessitate the evolution of novel solutions to the constraints, thus yielding a phenotype with higher fitness. The latter scenario may explain, for example, the evolution of complex retinotectal projections built on the "simplistic" morphology of the salamander visual system, imposed by the constraint of large cell size (reviewed in Roth et al., '97).

As for convergence and evolvability, recall the Waddington genetic assimilation experiments where a plastic ancestral population evolved rapid fixation of the *bithorax*-like phenotype under strong selection. As Schlichting and Pigliucci ('98) have noted, the plasticity of the ancestral population appears to have allowed for the rapid response to selection and that genetic assimilation likely represents a relatively common mechanism for phenotypic evolution. It is important to note here that the ancestral population apparently did not harbor genetic variation for the constitutive *bithorax*-like phenotype: it arose de novo in the course of selection. Whether convergent mechanisms are likely to arise out of such plastic systems by genetic assimilation has not been addressed. In addition, there are no available data on the frequency with which assimilation experiments are successful (since, presumably, negative results are not reported). Thus it is difficult to determine how common a mechanism for phenotypic evolution genetic assimilation may actually represent.

CONCLUSION

There appears to be a common misunderstanding in the literature about the definition of developmental constraints. My understanding of the term is that it does not represent prohibitions on the evolution of certain features, just that the developmental system is predisposed to respond to selection in a certain way. What exactly it is about developmental systems that lead to constraints is not known. I suggest that it is the modular nature of development that leads to such constraints, since these modules can be thought of (teleologically) as "easy" ways to solve new problems. Instances of convergence, by contrast, point to the absence of such constraints and may be characteristic of processes subject to phenotypic plasticity. Detailed investigation into the nature of parallelisms and convergences may represent the first step towards developing a predictive science of the mechanisms of evolution.

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Erratum

Hodin J. 2000. Plasticity and constraints in development and evolution. J Exp Zool (Mol Dev Evol) 288:1–20.

The sentences on page 8, in paragraph 2 of column 2 reads:

In the short-germ insects, typified by *Drosophila melanogaster*, all the segments are specified nearly simultaneously in a noncellular, syncitial environment (reviewed in Lawrence, '92). By contrast, in the long-germ insects, typified by the grasshopper *Schistocerca*, the segments are specified in a gradual anterior-to-posterior progression in a cellular environment (reviewed in Patel, '94; Tautz et al., '94).

The sentences should read:

In the long-germ insects, typified by *Drosophila melanogaster*, all the segments are specified nearly simultaneously in a noncellular, syncitial environment (reviewed in Lawrence, '92). By contrast, in the short-germ insects, typified by the grasshopper *Schistocerca*, the segments are specified in a gradual anterior-to-posterior progression in a cellular environment (reviewed in Patel, '94; Tautz et al., '94).

The terms "long-germ" and "short-germ" were reversed. The germ-type classification is key to the point being made in this paragraph: namely, showing that a similar-looking segmental plan can be formed by two rather different ontogenetic routes).

The author regrets the error.