Notes and comments

How can a character be developmentally constrained despite variation in developmental pathways?

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Abstract

A fundamental riddle of evolutionary developmental biology is the conservation of adult morphological patterns (Hall, 1992). Conservative patterns are either called body plans if they concern overall body design, or homologues if they concern parts of the body (Riedl, 1978; Roth, 1982; Sattler, 1984; Van Valen, 1982; Wagner, 1989a, 1989b). An adult pattern is considered conservative if it remains unchanged in spite of changes in function, as indicated by the original definition of homology by Owen, as a similarity of organs regardless of form and function (Owen, 1848). Conservation of anatomical features despite different adaptive pressures is naturally explained by developmental constraints (Wagner, 1986). However, this approach to explain the biological basis of homology is plagued by the fact that developmental pathways are often more variable than the characters that they produce (see Tab. 1) (Hall, 1992; Roth, 1988, 1991; Spemann, 1913; Wagner, 1989b). This is also true for any other application of the concept of developmental constraints. The widely held opinion that early stages of development are conservative because any early perturbation is likely to interfere with later development, is far from absolute, since a vast amount of data in comparative developmental biology speaks to developmental variation (see e.g. the examples in Tab. 1). The question then is, how can developmental constraints on adult variation be reconciled with the fact of developmental variation?
Table 1: Ten examples of variable development of homologous characters, where the variant developmental pathway does not have any substantial consequences for the adult pattern.

<table>
<thead>
<tr>
<th>Example</th>
<th>Reference</th>
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<tbody>
<tr>
<td>Meckel's cartilage is induced by different tissues in amphibians,</td>
<td>(Hall, 1984)</td>
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<td>birds and mammals</td>
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<td>The orbitosphenoid bone develops as a membrane bone in the Amphibia</td>
<td>(Bellairs and Gans, 1983)</td>
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<td>(Amphibia) while it is a replacement bone in other vertebrates.</td>
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<tr>
<td>The columella of the chick can be derived from ectomesenchyme</td>
<td>(Noden, 1983)</td>
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<tr>
<td>or mesodermal mesenchyme.</td>
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<td>The sequences of chronogenetic condensations in the limbs of Ambystoma</td>
<td>(Blanco and Alberch, 1992)</td>
</tr>
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<td>mexicanum and Triturus marmoratus are different.</td>
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<td>Lenses develop from ectoderm but regenerate from the iris in salamanders.</td>
<td>for ref. see (Goss, 1969)</td>
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<td>The body of insects develops in two very different modes in different</td>
<td>for ref. see (Raff and</td>
</tr>
<tr>
<td>In Drosophila the gene even-skipped plays a necessary role in</td>
<td>(Patel, et al., 1992)</td>
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<td>segmentation as a member of the class of a pair rule genes. In the</td>
<td></td>
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<tr>
<td>grasshopper (Schistocerca americana) the homologue to even-skipped has</td>
<td></td>
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<tr>
<td>no pair rule function in segmentation.</td>
<td></td>
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<tr>
<td>Directly developing anurans (Eleutherodactylus) differ from indirect</td>
<td>review in (Raff and</td>
</tr>
<tr>
<td>developing ones in their morphogenetic sequences but not in their adult</td>
<td>Kaufman, 1983)</td>
</tr>
<tr>
<td>morphology.</td>
<td></td>
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<tr>
<td>Directly developing sea urchins (Heliocidaris) differ from indirect</td>
<td>(Wray and Raff, 1989;</td>
</tr>
<tr>
<td>developing ones in their morphogenetic sequences but not in their</td>
<td>Wray and Raff, 1990; Wray and</td>
</tr>
<tr>
<td>Cell autonomous determination of the D quadrant cell in spiralian</td>
<td>(Freeman and Lundelius, 1992)</td>
</tr>
<tr>
<td>coelomates is derived from inductive modes of development without</td>
<td></td>
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<td>consequences for adult morphology.</td>
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</tbody>
</table>

In this paper we present a hypothesis that provides an answer to this question that can account for cases of moderate variation in the developmental pathway. It is suggested that developmental constraints are to a large extent due to epigenetic interactions late in ontogeny. If these late events are genetically decoupled from earlier developmental transformations, then variation early in development would have little effect on the adult pattern. The hypothesis is based on empirical studies on the anatomy, variation, regeneration and experimentally induced plasticity of blenny pectoral fin hooks (Brandstätter et al., 1990; Misof and Wagner, 1992; Wagner and Almeder, 1991; Wagner and Misof, 1992). Pectoral fin hooks are anatomical specializations that are adaptations to the bottom dwelling life style of blennies. Briefly, empirical work has established three facts:

1) The fin hooks are specializations of the lower fin rays, consisting of three characters (an asymmetric regression of the fin web, a connective tissue pad associated with the bony fin ray, called lepidotrichal cord (LC), and a
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Fig. 1. Schematic drawing of the first three fin rays of the pectoral showing the characters that constitute a pectoral fin hook in Salarias paro (Blenniidae, Perciformes). The fin is viewed from the inner side, i.e., the surface of the fin viewed faces the body when the fin is in resting position. The white structures phr are the bony fin rays, fn is the fin web, the black structure lc is the lepidotrichal chord (a connective tissue associated with the bony fin ray), and the stippled area cu is where the epidermis is expressing a cuticle. Note the correlated expression between fin web regression, lepidotrichal chord and cuticle.

cuticular specialization of the epidermis covering the LC (Brandstätter et al., 1990) (see Fig. 1). The expression of these characters is spatially correlated. The LC is most fully developed in the region of fin web regression and the cuticle is only found over the LC.

2) Regeneration experiments show that the expression of these characters is only loosely correlated, if at all, during histogenesis (Misof and Wagner, 1992). The sequence with which the characters appear during histogenesis is variable. For instance there are cases where the cuticle comes later than the LC, but there are also cases where a cuticle was found without a local contact to an LC. Similarly cases have been found where the LC and the cuticle were fully developed without any sign of fin web regression and vice versa. This variation in the histogenetic sequence is incompatible with the view that the fin hook characters are developmentally coupled. The stereotypic adult pattern appears only later in regeneration.

3) Perturbation experiments with adult fins show that the characters are mutually modulated and in part dependent on each others presence for their maintenance (see Fig. 2 for a summary). For instance the amputation of the tip of a fin ray at the site of fin web attachment leads to a reactive fin web growth during the regeneration of the fin ray tip (Wagner and Almeder, 1991). This is taken as evidence that the LC and/or the cuticle inhibits fin web growth, because during early stages of fin ray regeneration only bony fin
rays are formed and the LC and cuticle come later. In another series of experiments it has been shown that resection of the LC leads to the loss of the cuticle (Wagner, in preparation), which implies that the LC is necessary for the maintenance of the cuticle. The transplantation of the LC to non-cuticular parts of the fin never led to an ectopic cuticularization, even if the blennies are able to differentiate new hooks as adults. Finally, the transplantation of the LC to ectopic locations leaves the LC intact for up to eight weeks. This shows that the LC is autonomous and does not depend on the presence of the other characters in the fin hook, while the cuticle and the fin web regression do. Thus the adult pattern is maintained by constitutive epigenetic tissue interactions.

Together these three facts imply that the correlated expression of the characters in the adult is not due to morphogenetic, generative (e.g. inductive) interdependencies among them. Morphogenesis and histogenesis of the sub-characters are largely independent. The correlated expression is finally achieved by a secondary adjustment after the characters are established.

The distinction between generative processes and maintaining interactions is not confined to the fin hook example. Most obvious is the situation in neural development. There are three main generative processes during neural development: neurogenesis (generation of nerve cells), axon and dendrite differentiation, and synapse formation (for ref. see Jacobson, 1978). However, the range of connections and cells generated by these processes are not defining the adult structure of the brain. Not all of the cells, axons and synapses survive and get stabilized by cell-cell interactions. For instance many more motor neurons are generated in the chick spinal chord than finally make up the motor neuron pool of the adult spinal chord. The survival of a motor neuron depends on its ability to establish a functional
contact to a muscle cell. Hence, a stabilizing signal decides about the maintenance of the motor neurons. It is clear that the interactions that stabilize a spinal motor neuron are not the same cellular and genetic mechanisms that have generated the motor neurons in the first place. Similarly, the structural maintenance of newt limb depends on the presence of innervation, while morphogenesis of the limb is nerve independent (for ref. see Goss, 1969). Recently it was found that the Steel gene locus of mice codes for a growth factor that is necessary to maintain melanocyte precursor cells but is not involved in melanocytic differentiation (Murphy et al., 1992). It seems that the distinction between generative and maintaining processes is a common phenomenon.

Our hypothesis to reconcile developmental constraints with variable developmental pathways is a generalization of these experimental results. The results suggest that morphogenetic and histogenetic mechanisms are (at least partially) decoupled from the mechanisms that determine which anatomical pattern is stable. We suggest that it is necessary to distinguish between generative morphogenetic mechanisms and stabilizing “morphostatic” mechanisms. If the stabilizing mechanisms are affected by other genes than the generative processes, then developmental pathways can vary while the adult pattern remains the same. The adult pattern would be determined by the stabilizing mechanisms as long as variation of the developmental process provides the necessary material for stabilizing the adult configuration. Of course, the stabilizing influence of morphostatic mechanisms does not allow boundless variation of the developmental pathway. Only those variations of development would be permitted which provide the necessary cellular material at the right spot and the right time. Within this frame, the developmental pathways can have many degrees of freedom. For instance, it does not matter where the cells originated as long as they are able to interact with other cells to build and stabilize the particular character (e.g. the cells that come from the iris to regenerate a lens in salamanders). The prediction is that the developmental variation observed among species covers the range of variation that is compatible with a conserved system of morphostatic interactions.

The relationship between constrained adult variation and broad developmental variation becomes clearer if two kinds of developmental constraints are distinguished:

**Generative constraints** (= developmental constraints in the narrow sense) are limitations to phenotypic variation caused by the properties of the generative process of development.

**Morphostatic constraints** are limitations to phenotypic variation caused by the stabilization of particular patterns rather than the inability of the generative processes of development to produce them in the first place.

Both are developmental constraints in the sense of Maynard Smith and co-workers (1985), for they cause biases in the production of phenotypic variation, but they differ in the ontogenetic stage at which the relevant epigenetic interactions occur.
Generative constraints arise from interactions during morphogenesis and morphostatic constraints arise from regulatory interactions maintaining the characters later on.

But it is biologically plausible to assume two genetically decoupled classes of mechanisms, generative and stabilizing ones, in general? In the case of the pectoral fin hook system the biological reason for the presence of a stabilizing mechanism in addition to a generative system is quite obvious. Fin hooks are developed during late stages of larval development in blennies (Pazmándi, unpublished observation), when the fin is only a few millimeters long. In adults the same fin is about 10 times larger, but still has the same characteristic arrangement of tissues. Consequently the cells constituting fin hooks of an adult can hardly be the same as those that initially established the fin hook in the larvae. During fin growth, turnover of the tissues needs to be regulated to maintain the functionally important arrangement of tissues, thus to preserve the anatomical configuration. The stabilizing mechanisms thus play a different biological role than the generative, histogenetic processes. However, as soon as the stabilizing mechanisms are in place during evolution, the expression of genetic variation will be canalized by the action of the stabilizing mechanisms. Developmental stabilization implies partial decoupling of adult variation from variation in developmental pathways.

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