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Ancestors and variants: tales from the cryptic

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SUMMARY Those who work at the interface of development and evolution are united by the conviction that developmental comparisons can shed light on both the evolution of specific morphologies and the macroevolutionary process itself. In practice, however, the field comprises a diversity of approaches. As the field grows and practitioners attempt to digest a growing mountain of comparative data, the various approaches of "Evo Devo" have themselves evolved. A meeting organized by the authors and held at the University of Chicago in the Spring of 1999 illustrated some of these changes. This review will draw on its content to discuss recent developments in two areas: the reconstruction of common ancestors and the developmental basis of evolutionary change.

DIGGING FOR THE ROOTS: RECONSTRUCTING ANCESTORS

Comparative developmental work over the last two decades has revealed striking similarity in the developmental processes of widely divergent taxa. Perhaps the most prominent example is the possession of clustered *Hox* genes and their colinear expression patterns across the Bilateria. This conservation may reach even further down the metazoan tree than previously appreciated. Using the sea anemone *Nematostella*, John Finnerty presented evidence for linked Cnidarian *Hox* genes, and for Cnidarian *ParaHox* genes (Finnerty and Martindale 1999), members of the putative evolutionary sister to the *Hox* cluster (Brooke et al. 1998). We anxiously await expression data for these radial organisms, because they may well provide clues regarding the ancestral functions of these ancient genes.

More recently, developmental similarities have been used to assert the homology of structures traditionally thought to have evolved independently, meaning that the structures were derived from a similar structure possessed by the common ancestor. In what is perhaps the most ambitious example of this approach, developmental similarities between protostomes and deuterostomes have been used to posit a common ancestor to these groups whose morphological complexity (including segments, a heart-like organ, outgrowths, and primitive photoreceptors) surpasses many previous reconstructions (review by Knoll and Carroll 1999). Recent molecular phylogenies have encouraged this notion by removing groups with relatively simple morphologies such as nematodes and flatworms from the base of the bilaterian tree and recasting them as products of regressive loss (review by Adoutte et al. 1999). However, a closer analysis of acoel flatworms suggests that acoels themselves may branch deeper than the split between protostomes and deuterostomes (Ruiz-Trillo et al. 1999). That acoels are indeed developmentally very different from other flatworms was underscored by Jonathan Henry, who presented a cell lineage analysis suggesting that at least one member of this group lacks ectomesoderm, a hallmark of the classic spiralians including other flatworms (Henry et al. 2000). Henry also commented on the difficulty of deriving the "duet" spiral cleavage pattern of acoels from the "quartet" spiral cleavage exhibited by other flatworms (polyclad turbellarians) and other spiralians. To the extent that living organisms can be used to infer ancestral states, this basal placement of the acoels represents a potential challenge to the conception of a more complex protostome–deuterostome ancestor based on comparative developmental work (but see Knoll and Carroll 1999).

Do we find evidence for such a complex ancestor in the fossil record? Strictly speaking the answer is no, since the few bilaterian fossils known from pre-Cambrian deposits lack such complexity (see Valentine et al. 1999). However, Douglas Erwin pointed out that the plausibility of a complex ancestor depends to a large extent on when we think it lived, and thus on the timing of the protostome–deuterostome split. In particular, early estimates of the divergence time ($>$1000 Ma) are problematic for a complex bilaterian ancestor, because we would expect fossil evidence of its complex descendents to have been found somewhere in deposits spanning the lengthy interval leading up to the base of the Cambrian (543 Ma). In contrast, later estimates of the divergence time (approximately 600 Ma) are more consistent with a complex ancestor (and the fact that we have yet to find de-

finitive fossil evidence for it), because its descendents would have existed for a relatively short interval prior to the Cambrian. The alternative hypothesis, of course, is that the common ancestor of protostomes and deuterostomes lacked the morphological complexity we might predict based on developmental similarities.

With the assertion of a simple ancestor—and the implication that similar structures in protostomes and deuterostomes evolved independently—comes the onus of explaining how the same genes have been independently recruited to function in the development of similar structures. For example, if the hearts of *Drosophila melanogaster* and vertebrates evolved independently, how did *tinman* and the homologous *Nkx* genes, respectively, come to pattern these structures? Answers to questions such as this will surely tell us much about the evolutionary process, and, importantly, help us to understand the ways in which development impinges on evolutionary change.

THE DEVELOPMENTAL BASIS OF EVOLUTIONARY CHANGE

How does development influence evolutionary change? One early idea was the concept of developmental constraint, in which an integrated developmental system limits the range of novel variants upon which natural selection can act (Schwenck 1994/95). This concept is part of a larger attempt to understand changes in adult morphology by investigating the underlying changes in development. More recently new tools have made available a variety of experimental approaches, many of which were highlighted during the meeting. Using gene expression to probe the identity of nervous system and mesodermal structures in sea urchins, Rudy Raff discussed the transformation from bilateral to radial symmetry in the lineage leading to echinoderms. While nerve radii lack *Hox* expression, these tissues do express the anterior neural marker *Otx*, suggesting that for echinoderms the evolution of radial symmetry involved a loss of trunk and tail coupled with modification of anterior structures.

Other presentations attempted to identify the developmental changes responsible for observed changes in morphology. Billie Swalla discussed the roles of the *manx* and *bobcat* genes in two closely related species of ascidians whose larvae differ in the possession of a tail (Swalla and Jeffery 1996; Swalla et al. 1999). In the species that lacks a tail, *manx* mRNA is downregulated during oogenesis, while *bobcat* mRNA persists. Anti-sense RNA experiments, however, demonstrate that both gene products are required for proper tail formation. Thus, repression of at least one of these genes may have been a critical step in the evolution of larval taillessness.

As the ascidian case illustrates, the ability to test the function of genes in a diversity of organisms is an essential component of our attempt to understand their role in both development and evolution. For example, expression patterns of the *Hox* gene *proboscipedia* (*pb*) have been shown to vary among insects, but the significance of this variation has remained obscure. Thom Kaufman described the result of interfering with *pb* function in the milkweed bug, *Oncopeltus*. Injecting double-stranded RNA (RNAi) for this gene into embryos produced a homeotic phenotype similar to the *Drosophila pb* mutant. The capacity to interfere with *pb* function in a range of "nonmodel" insects may well open the door to an analysis of the functional significance of the observed variation in expression pattern. Nevertheless, as long as we follow the opportunistic, albeit fruitful, strategy of pursuing candidate genes, we run the risk of filtering diversity through the lens of *Drosophila*. How much of the evolutionary picture do we miss when we cast a net made for catching flies?

An important advance has come from the application of quantitative trait loci analysis to polymorphisms within single plant and animal species, as well as to traits that vary between closely related species. These studies are beginning to identify loci involved in the evolution of traits such as floral morphology (Doebley et al. 1997; Bradshaw et al. 1998), as well as bristle pattern and genital morphology in fruit flies (Long et al. 1995; True et al. 1997). While quantitative trait loci analysis will undoubtedly shed light on the adequacy of the candidate gene approach, it should be noted that this technique is only suited to the study of variation within single or closely related species. When considering a specific trait, it is not at all clear that the loci responsible for variation between closely related organisms can fully account for variation between distantly related organisms. Regardless, and despite the successful use of these techniques in identifying isolated evolutionary steps, a question remains: Do we really have the tools to discover the actual sequence of genetic changes that culminate in the evolution of morphology?

A large piece of the puzzle is undoubtedly the origin of genetic variation. Given that most mutations of functional significance are likely to be detrimental, how are we to understand the fixation of variants within populations? As a possible answer, Martin Kreitman proposed a model for the accumulation of variation within *cis*-regulatory systems. The model is based on intra- and interspecific differences in the portion of the *even-skipped* enhancer responsible for the second of seven stripes which pattern the *Drosophila* embryo (Ludwig et al. 2000). The model proposes changes through accretion, in which transcription factor binding sites, by virtue of their small size, arise frequently in populations through mutation. Of the novel sites, those that bind transcription factors with weak affinity are likely to have a relatively small impact on enhancer function. Despite strong maintenance selection for the second stripe of *even-skipped*, these weak sites may become fixed by genetic drift.

Surprisingly, this "cryptic variation," which under normal circumstances has no affect on phenotype, is not relegated to

the realm of fine-scale genetic changes. An interesting case in this regard was presented by Marie-Anne Felix, who described the network of cell interactions responsible for patterning the vulva in different species of soil nematodes (review by Felix 1999). These comparative studies have revealed a remarkable amount of developmental variation in vulval patterning, including the role of the anchor cell, the number of induction events, and the role of signals from the gonad. While the underlying developmental mechanisms have proved quite labile, the vulval morphology has remained remarkably static. Understanding the source of such extensive variation, especially when that variation appears to be hidden from selective forces, is surely a challenge. One interpretation of these data is that the observed developmental variation, like the variation in transcription factor binding sites, results from the interplay of genetic drift and the compensatory changes produced by maintenance selection, this time for a functional vulva.

An intriguing possibility is that this cryptic variation may provide the raw material for evolutionary change. Rather than being entirely irrelevant with regard to vulval phenotype, this developmental variation may manifest itself as phenotypic variation in certain genetic or environmental contexts. An interesting example of the role of genetic and environmental factors in revealing cryptic variation comes from a recent paper by Rutherford and Lindquist (1998). This study demonstrates that genetic or environmental inactivation of the *Drosophila* chaperone protein Hsp90 exposes extensive variation in the form of strain-specific phenotypes. Evidently this protein has allowed for the accumulation of normally silent variation. Upon exposure by particular genetic or environmental perturbations, such pre-existing variation may well prove to be important raw material for natural selection. Understanding the complex interplay of genetic and environmental factors involved in morphological evolution is a daunting task. As our approaches evolve and data accumulates, however, we may soon be better equipped to make the jump from the patterns of developmental variation to the process of morphological evolution.

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