Developmental Genes and the Reconstruction of Metazoan Evolution—Implications of Evolutionary Loss, Limits on Inference of Ancestry and Type 2 Errors

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SYNOPSIS. We consider three issues that appear to be important in the interpretation of developmental genetics in an evolutionary context. The three issues under discussion are 1) evolutionary loss as applied to evo-devo data; 2) the limits on our ability to infer ancestry based on tree reconstruction; and 3) “type 2” errors in the assessment of homology of developmental gene expression data. Lack of consideration of any or all of these disparate issues narrows the set of hypotheses under consideration. We examine these issues through examples drawing on new data on POU domain genes as well as through reference to published work on Distal-less, engrailed and Nk2 genes.

INTRODUCTION
The reconstruction of metazoan evolution at the phylum level has proved difficult despite over a century of interest in the problem. It has been generally hoped that the application of molecular techniques to phylogenetic analysis and comparative development would reveal the relationships between different higher taxa and the mechanisms of body plan evolution that generated novel metazoan form. Substantial progress has been made with these new techniques, but many issues have yet to be resolved. Here we examine three disparate issues that limit the investigation of the evolution of development as currently practiced. In the absence of consideration of these issues, the range of data available for interpretation and, perhaps more importantly, the set of hypotheses under examination tends to narrow. Thus, although the issues considered are distinct from one another, they are linked in their potential to narrow the field of discussion. Given that the evo-devo discipline is not mature, and that a limited number of taxa have been examined in comparative studies, we feel a broad range of hypotheses need to be scrutinized. In a sense we argue for the application of the doctrine of “multiple working hypotheses” that long held sway in the exploratory phase of geologic investigation (Chamberlain, 1890). This approach to science considers all rational hypotheses on equal footing, and can be distinct in application from the single hypothesis and null frequently seen in the typical thesis proposal. The three issues under discussion are 1) evolutionary loss as applied to evo-devo data; 2) the limits on our ability to infer ancestry based on tree reconstruction; and 3) “type 2” errors in assessment of homology of developmental gene expression. In our discussion of these points we draw on examples from the developmental genetic literature and from our own work.

“DEGENERACY” LOSS AS AN IMPORTANT COMPONENT OF EVOLUTION
E. Ray Lankester initially applied the term degeneracy to evolution in 1879 in his presentation to the British Association for the Advancement of Science:

“. . . Any new set of conditions occurring to an animal which render its food and safety very easily attained seem to lead as a rule to degeneration; just as an active healthy man sometimes degenerates when he becomes suddenly possessed of a fortune; or as ancient Rome degenerated when possessed of the riches of the ancient world.”

Lankester (1890) went on to clarify these colorful analogies by referring to parasites and sessile filter-feeding marine invertebrates, such as sponges and barnacles, as examples of forms that had degenerated and lost features that support an errant mode of life. Consistent with this line of reasoning he viewed ascidians as reduced chordates that retained a vestige of the chordate structure in their larvae. Today, many follow Garstang’s (1928) arguments that the chordate body plan is an elaboration of the larval structures of ascidians and that the ascidian larval notochord is the novel evolutionary feature from which chordate features are derived. Viewed broadly, Lankester’s invocation of degeneracy was a call to consider a non-progressive component of evolution, especially in the context of novel body-plans and higher taxa. It is the evolution of such divergent morphology that still challenges us today, and this is the area where many hope the use of developmental genetic data will continue to yield rapid advances.

The conservation of gene sequences and functions in general, and in developmental genes in particular, has only become evident in the last couple of decades. Such conservation is not consistent with the neo-Darwinian view developed by the new synthesis workers in the middle part of the 20th Century. In the new synthesis perspective genes were plastic and interchangeable over long evolutionary time scales. In this older context, gain and loss of gene function in development...
was not of special interest since the genes themselves were not viewed as comparable features shared by divergent higher taxa. Now that it is evident that genes are often conserved, the potential influence of loss of genes, as well as loss of specific gene functions, becomes of more critical interest. We now combine Lancaster’s caveat with a realization that loss of morphology likely correlates with loss of developmental processes, gene functions and even genes themselves. Despite the likely importance of degeneracy or loss in the evolution of development, and the fact that it is axiomatic that loss is easier than gain in evolution, loss has been virtually unexamined in the evolutionary interpretation of developmental data.

Mechanisms of loss

Presumably morphologic loss could be accomplished through a number of developmental mechanisms. Some of these changes could involve many genes of small effect (along the lines of the expectations of the new synthesis). However, where crosses between morphologically distinct “taxa” are possible, such as between teosinte and maize (Doebly et al., 1997), or within the Stickleback Gasterosteus aculeatus complex (Peichel et al., 2001), the data suggest that one or a few genes of major effect are responsible for substantial changes in morphology. These data, in conjunction with comparative developmental genetic studies, support the focus on a small number of candidate genes in studies of morphologic change. In this context, the loss of components of gene function and complete loss of entire regulatory genes are two mechanisms by which loss at the genetic level could influence morphologic evolution. First we reconsider the expression data for the gene engrailed in the context of loss of components of gene function in evolution, and then discuss the POU gene Pit-1, and the Nk2 gene family in the context of evolutionary loss of developmental regulatory genes.

Loss of components of gene function with reference to engrailed

The gene engrailed is known to have several discrete functional components in Drosophila development including wing, leg, hindgut and neural patterning. Nevertheless, the potential role of the engrailed gene in segmentation (segment polarity) initially described in Drosophila by Nüsslein-Volhard and Wieschaus (1980) has been the focus of comparative studies. This focus reflects long-standing interest in potential homology of repeated or segmental structures found in arthropods, annelids, molluscs and vertebrates, amongst other taxa (e.g., DeRobertis, 1997).

Across the arthropods engrailed expression is associated with segment boundaries supporting homology and monophyly of arthropod segments, and Hox gene expression appears to support homology of a repeated set of features along the A/P axis across the Bilateria. Nevertheless, the use of engrailed expression to identify sets of serially repeated homologous features across the Bilateria and even between protostome phyla has proved equivocal. In this context, the absence of engrailed stripe expression in the ectoderm of Onychophora is particularly interesting (Wedeen et al., 1997). Onychophora, also known as velvet worms or lobopods, are considered segmented (e.g., Anderson, 1973) and close relatives of the Arthropoda. Thus as soon as one departs from the arthropods the association between segmentation and engrailed striping of the ectoderm appears to break down. This absence is all the more striking as posterior limb expression comparable to that found in arthropods is present. We argue that the absence of segmental ectodermal expression in Onychophora can be better understood by focusing on the potential function of the ectodermal component of engrailed expression in bilaterian invertebrate skeletogenesis rather than strictly in segmentation. This approach requires consideration of the potential for loss of this functional component, while other functions such as that in limb development are retained.

Consideration of evolutionary loss of components of gene function and the retention of the gene in other conserved functions yields alternative evolutionary interpretations from previous approaches that do not formally consider loss. Here we discuss a skeletal-binding role for ectodermal engrailed expression. Given loss of components of gene function, this hypothesis seems consistent with much of the available data. We advocate this working hypothesis while recognizing that “multiple working hypotheses” merit consideration. Alternative and related hypotheses include those that deal explicitly with engrailed in mesodermal units, chordate evolution (Holland et al., 1997), and terminal posterior addition of iterated elements in development (Jacobs et al., 2000). Loss of components of gene expression or aspects of development appear critical for some of these other hypotheses as well.

In molluscs, engrailed expression surrounds the shell gland of snails (Moshel et al., 1998) and scaphopods (Wanninger and Haszprunar, 2001). It also surrounds and bounds each of the plates of chitons (Jacobs et al., 2000). In the absence of other data, these observations could be viewed as a consequence of the reduction from a metamerically repeated chiton-like ancestor to a monomeric chonchiferan. However, other molluscan data are not consistent with a simple interpretation of segmental reduction or segment fusion. In the chiton, expression surrounds each of the calcareous spicules in the girdle as well as surrounding each of the plate fields. During bivalve development engrailed expression surrounds both values including the intervening hinge (Jacobs et al., 2000). These observations suggest that the expression patterns are not dictated by, or correlated with, some overarching segmental bauplan. Rather ectodermal engrailed expression appears closely linked with, and surrounds, the sets of cells in the ectoderm that produce molluscan shell material. This leads to the argument that one shared func-
A re-examination of ectodermal aspects of *engrailed* in arthropods reveals that expression correlates with the flexible segmental boundaries in the skeletal ectoderm in a manner consistent with our working hypothesis. In addition, the arthropod head does not have complete circumferential expression of *engrailed* (Rogers and Kaufman, 1996), perhaps because the segmental boundaries do not form discrete joints in the cephalic exoskeleton. Furthermore, in crayfish (Patel et al., 1989) *engrailed* expression parallels the anterior/posterior axis between the dorsal and ventral elements (sternite and tergite) of the last abdominal segment. This stripe of expression is not at a segmental boundary—it likely demarcates a zone of flexibility in the exoskeleton required for the tail flipping behavior characteristic of these crustaceans. Thus, even in arthropods it can be argued that ectodermal *engrailed* expression may be patterning boundaries between skeletal units, not all of which are segmental.

A skeletal unit bounding function for ectodermally expressed *engrailed* appears consistent with much of the data available from non-arthropod taxa. For example, chaetal sacks are one of the few ectodermal features of polychaetes that regularly express *engrailed* (Seaver et al., 2000), and chaetae are the primary indurated elements in the ectoderm. Furthermore, the absence of *engrailed* expression in the onychophoran ectoderm makes more sense in the context of a skeleton bounding function rather than a general segmentation program. Living Onychophora lack an ectodermal skeleton (Storch, 1984). The absence of *engrailed* expression in the ectoderm of these organisms then appears to be the result of loss of this component of gene function. This interpretation is reasonable in light of the fact that Cambrian and Ordovician fossil lobopods were skeletonized bearing dermal elements above the limb on each metameric unit (e.g., Ramskold and Hou, 1991; Dzik, 1993).

Thus the data support an exoskeleton or shell bounding function for *engrailed* in modern molluscs, arthropods and possibly polychaetes. Despite their obvious differences, both mollusc shells and arthropod cuticle contain chitin (Weiner and Traub, 1980). Given a bias toward loss in non-skeletonized taxa such as Onychophora, the available data support ancestry of a skeletal unit bounding function of *engrailed* in protostomes. It has also been stated that ectodermal *engrailed* expression “bounds” the regions that produce ossicles in the developing brittle star. Although the ossicles are mesodermally derived rather than ectodermal, there is a similarity in the skeletal bounding aspect of the ectodermal component of *engrailed* expression in these echinoderms (Lowe and Wray, 1997). This bounding of skeletal elements by ectodermal *engrailed* expressing cells in both deuterostome and protostome development compels one to consider the possibility of a common origin of skeletogenesis across the bilaterian invertebrates. Presumably vertebrate skeletons evolved secondarily.

An origin of skeletons near the base of the Bilateria has important implications for the Cambrian radiation. Many workers presume that Cambrian radiation was in part precipitated by the evolution of skeletons in association with, and in response to predation. In many systems skeletal armor has been correlated with the level or type of predation pressure in the environment. For example, *Daphnia* and stickleback evolve spiny armor in the face of engulfing predation (e.g., Peichel et al., 2001) and similarly, selection produces thicker snail shells in the face of crab predation. Tardigrades, close relatives of Onychophora and Arthropoda, possess ornamented armored exoskeletons in high predation marine environments. Terrestrial tardigrades are free of such armor (e.g., Kinchin, 1994). Given current data, the *engrailed* gene is exclusive to the Bilateria and seemingly associated with bilaterian skeletogenesis. Perhaps the integrated evolution of bilaterian skeletons and increasing predation at the base of the Cambrian had a causal role in the phylogenetic radiation and trophic diversification of Cambrian animals. The point in time where the *engrailed* gene became involved with a skeletogenetic process would then also be a component of this causal nexus. This hypothesis deserves further test—it predicts *engrailed* expression bounding skeletal features in other bilaterian invertebrates, such as brachiopods where expression has yet to be assessed.

Given the above, selective and adaptive scenarios for evolutionary loss of exoskeletons seem relatively straightforward. However, we have to explain the loss of one function of the gene while others are retained. The “cassette or module” model of promoter evolution advocated by Davidson and others (e.g., Yuh et al., 2001) provides a potential avenue for loss of components of gene function. If regions of the promoter composed of suites of binding sites are separated into cassettes based on their function, then simple deletion could generate rapid loss of one cassette and one gene function with minimal impact on others. Alternatively, if promoter control of different aspects of gene expression is not spatially separated, selection could easily eliminate those binding sites required for a particular function. Thus, disassociation and mosaic evolution of gene function with loss of particular functions appears mechanistically plausible and could be an important aspect of evolution. Ideally, one could identify sets of binding sites that are specific to particular aspects of gene function such as the nerve, limb and segment/skeletal boundary functions evident in *Drosophila engrailed*, and compare their presence either through sequence analysis or transgenics. As sequence analysis of promoter regions has proved difficult across distantly related taxa, an ideal study would compare closely related taxa with and without a given property. Alternatively, transgenic studies may prove to be of surprising utility in comparing the promoter function of relatively distant taxa as introduction of
genes has been shown to rescue gene function in very divergent taxa where promoter analysis based on sequence might be difficult.

**Gene loss with reference to Pit-1 and Nk2 genes**

Many workers have emphasized the potential importance of gene and genome duplication in the evolution of novelty and reconstruction of evolutionary pattern and process. However, gene loss has received little attention in the evo-devo field, despite its potential to dramatically change evolutionary interpretation. We have explored POU/homeodomain regulatory genes in basal metazoans. Through the course of this work it became apparent that one of these genes is present in vertebrates, jellyfish, ctenophores and sponges, but absent in the fly and nematode model systems. The gene in question, Pit-1, is critical to pituitary development and our observation of it in more basal taxa will likely lead to a different interpretation of the evolution of the pituitary. We use this example, as well as the absence of several members of the Nk2 class of homeodomains in nematodes, to illustrate some of the evolutionary implications of developmental gene loss.

In our work on the POU homeodomain genes we conducted a PCR survey of a range of basal metazoan taxa (unpublished data). These genes are a subset of homeodomain DNA binding developmental regulatory genes that also contain a POU DNA binding domain. Our initial interest was in a gene we refer to by its vertebrate name Brain 3 (also known as POU class IV) which appears to be involved in the development of all sense organs. However, our survey also recovered two other genes, a Brain 1 (POU class 3) and Pit-1 (class 1). The recovery of the Pit-1 gene is of particular interest as it had previously been recovered from chordates and had reasonably been interpreted as a new gene associated with the evolution of the pituitary. The pituitary itself is presumed to be an exclusively vertebrate organ with antecedents going back only as far as basal chordates. Hatscheck’s pit in amphioxus is strongly implicated as sharing ancestry with the vertebrate pituitary, and a structure in the ascidian pharynx has been more tentatively identified as a pituitary homologue. No pituitary homologues have been established in other invertebrates, and Pit-1 is a vertebrate homologue. No pituitary homologues have been identified as a pituitary homologue. No pituitary homologues have been established in other invertebrates, and Pit-1 is absent from both *Drosophila* and the nematode *Caenorhabditis*. This is negative evidence and under normal circumstances the proving of a negative is difficult. However, the genomes of both these organisms have been sequenced and searches do not recover this gene. Given this absence, co-evolution of Pit-1 and the pituitary near the base of the chordate clade seemed reasonable. Thus we were surprised when we recovered Pit-1 sequence from multiple, ctenophore, cnidarian and sponge lineages. In the context of these new sequences one previously reported sponge sequence is now recognizable as Pit-1 (Seimiya et al., 1997). Apparently, Pit-1 evolved at the base of the Metazoa and was subsequently lost in one or more protostome lineages, suggesting the intriguing possibility of evolutionary antecedents of the pituitary in basal Metazoa.

Scenarios of pituitary evolution traditionally focused on the base of the chordates consistent with the exclusively chordate nature of the pituitary structure. Data from amphioxus and vertebrate development suggest that the adenohypophyseal portion of the vertebrate pituitary was once an external sense organ that has been internalized in the chordate lineage where it now has an endocrine function coordinating reproduction. Thus the evidence suggests that the evolutionary antecedent of the vertebrate pituitary was an external sensory structure—possibly involved in reception of signals required to synchronize reproduction. Such a structure would likely be most effective in the marine realm where chemical and light signals often play a pervasive role in coordinating reproduction. Such signaling appears to be quite ancient as deeper branches of eucaryotes, such as marine algae, use such signals to coordinate reproduction. Thus reproductive signaling may have preceded and contributed to the evolution of sensory and neural functions in Metazoa.

Given the deep ancestry and potential critical function of the Pit-1 gene, absence of the gene in the genome of flies and nematodes comes as a surprise. However, several competing scenarios could explain this loss. Signaling systems mediated by other non-pituitary related sensory structures may be more suited to the terrestrial environment. Here the pheromone sensitivity of moth antennae provides an example. Thus evolution of terrestrial forms could precipitate pituitary loss in the Arthropod and nematode lineages. In this scenario co-option of the pituitary for an internal endocrine function protected the pituitary from loss in the face of a changing environment in the evolution of terrestrial vertebrates. Alternatively, as both nematodes and arthropods are currently thought to be members of the Ecdysozoa, loss of this gene could be associated with, or might even have been precipitated by, the evolution of molting in this group. Lastly, this lack of Pit-1 gene could be a product of a loss basal in the protostomes, an interpretation consistent with our inability to recover Pit-1 from molluscs, annelids and flatworms. Until we have a better understanding of the distribution of these genes across a range of taxa, we will not know the evolutionary context in which the loss of this gene occurred.

The Nk2 gene family provides another example of evolutionary loss of developmental genes. In *Drosophila* there are four genes that are related to the four gene subfamilies in vertebrates where multiple copies of regulatory genes is the norm. These subfamilies include; a) *bagpipe* where fly and vertebrate genes share aspects of function in mesoderm development; b) *tinman/cardiac* genes that function in mesodermal patterning and are specific to heart development in flies and vertebrates; c) *vnd/Nk2.2* genes that function in the midline patterning of the nervous system of flies and vertebrates; and d) *scarecrow/ttf/Nk2.1* which has a thyroid function in vertebrates and is expressed in...
the pharyngeal region of Drosophila. Given these similarities it appears that 4 genes were present and had already evolved a certain degree of functional specialization in the common ancestor of vertebrates and flies (Jacobs et al., 1998; Holland et al., 2003).

Given the view that nematodes group with arthropods in the Ecdysozoa, the presence of only one Nk2 homologue in Caenorhabditis suggests gene loss in a portion of the nematode lineage internal to the Ecdysozoa. On the basis of sequence similarity and tree reconstruction the remaining gene appears to be a homologue of vnd/Nk2 gene. However, the mutants and developmental expression of the nematode gene suggests functions similar to the tinman/cardiac genes (Huan et al., 1998), not the vnd-like neural functions that one would infer from tree topology. These conflicts between sequence and functional interpretation may result from a co-operation or transfer of gene function to remaining genes in association with gene loss. As stated previously, gene duplication has often been implicated in the evolution of gene function. Implicitly gene loss may also trigger evolution of gene function including the "reassignment" of gene function between closely related genes upon the loss of family members.

From the above discussion it should be clear that an awareness of the potential for evolutionary loss of both components of developmental gene functions and specific developmental genes yields a broader suite of evolutionary scenarios and hypotheses worthy of consideration.

LIMITS ON OUR ABILITY TO INFERENCE ANCESTRY WITH REFERENCE TO CNIDARIAN MEDUSAE

We now turn to a separate theme regarding the application of tree reconstructions to the interpretations of ancestry of characters and polarity of character evolution on trees. In the study of evolution and development much emphasis has been rightly placed on the presentation of data in the context of "trees"—supported hypotheses of relationship of the taxonomic units under discussion. This is all to the good except that reconstruction of character evolution on trees can easily be interpreted with excessive confidence, masking ambiguities of the evolutionary interpretation and consequently excluding evolutionary scenarios from consideration. Hence, we include this treatment to further highlight features that tend to narrow discussion and prematurely exclude working hypotheses from consideration. To this end we present one example involving the appropriate reconstruction of the medusa character state in cnidarian trees.

Small ribosomal subunit (18S) phylogenics place Anthozoa the class of Cnidaria lacking medusae, as the basal branch of living cnidarians (e.g., Collins, 2002). This tree topology led a number of workers to premise their work with the assumption that a polyp, but not a medusa, was present in the life history of the ancestral group. We argue that this premise is not supported. When considering characters unique to a group, and where the basal branch in the group lacks a character state typical of the rest of the group, then the ancestral condition of the group will be ambiguous. This ambiguity is accentuated if the character can not be legitimately coded in outgroups.

Ambiguity in character reconstruction can be formally demonstrated using Fitch Parsimony (Fitch, 1971). In Fitch's approach (as detailed on Fig. 1) one proceeds downward from the termini of the tree applying simple rules from set theory to determine the character states at each node. As two branches join at a node the intersection of the character states at the upper ends of the two branches is taken as the character state at the node. If that intersection is a null set, then the union of the two sets is used to generate the condition at the node. Proceeding down the tree, each node successively becomes the terminus of a more basal branch and, upon successive application of the rules, the condition of all nodes on the tree can be defined. For example, if two branches have the character state "p" on their terminal ends the intersection will be "p" and the character state "p" can be inferred at the node. However, if the two branches that come together at a node have different character states, "p" at the end of one branch and "a" on another, then the intersection will be a null set 0. The union of the two sets is used to generate the condition at the node. This is the case at the base of the Cnidarian tree where application of these simple rules documents the ambiguity of the presence or absence of a medusa stage in the life history. Given the lack of relevant outgroup data, the presence or absence of a medusa stage at the bottom of the tree gives equally parsimonious interpretations.

**FIG. 1.** *Fitch Parsimony and Character Reconstruction:* In Fitch's approach one proceeds downward from the termini of the tree using simple rules from set theory to determine the character states at each node. As two branches join at a node the intersection of the character states at the upper ends of the two branches is taken as the character state at the node. If that intersection is a null set, then the union of the two sets is used to generate the condition at the node. Proceeding down the tree, each node successively becomes the terminus of a more basal branch and, upon successive application of the rules, the condition of all nodes on the tree can be defined. For example, if two branches have the character state "p" on their terminal ends the intersection will be "p" and the character state "p" can be inferred at the node. However, if the two branches that come together at a node have different character states, "p" at the end of one branch and "a" on another, then the intersection will be a null set 0.
Although the ambiguity in the Fitch parsimony reconstruction at the node will not be eliminated, some constraints on the interpretation of character evolution are often imposed on the basis of the character state reconstructed on nodes that are more basal to the one in question. These in turn require reconstruction of the character in more basal branches. However, as we argued above, considerations of the presence and absence of polyps or medusae is only merited given the cnidarian condition, and living outgroups lack the cnidarian features that would permit aspects of cnidarian life history to be recognized and coded as characters. The fossil record provides a potential source of outgroups or “stem group” information for “crown-group” cnidarians (cnidarian taxa with living representatives). Fossil cnidarians include “medusoid” forms of Precambrian age. Nevertheless, the lack of preservation of life histories, the presence of a range of non-radial potentially diploblastic forms, and the lack of well understood relationships of these fossil taxa to modern Cnidaria, limit our ability to characterize the presence or absence of medusae in the stem of the cnidarian clade.

The above example explores just some of the potential sources of ambiguity in reconstructing character evolution on trees. Thus it is critical to consider the support for both tree reconstruction and character interpretation before excluding potential evolutionary scenarios from consideration.

**Type 2 Errors in the Assessment of Homology**

In evolution and development many important issues turn on questions of homology. Homology is defined as the presence in different organisms of a shared or similar feature due to derivation of that feature from a common ancestor. Here we wish to consider not just the hurdles that a hypothesis of homology must overcome to be accepted, but also under what conditions a hypothesis of homology can be formally rejected and in essence removed from further consideration. Again our concern is that working hypotheses may be prematurely eliminated from discussion.

Many in the evo-devo field appear to feel that it serves the discipline well to set a high standard for the acceptance of homology. Often if such a standard is not met then the statement comes out, either in a scientific report itself, in the secondary literature or in casual discussion at meetings, that the entities compared are not homologous. Such denials seem to advocate rigorous science, but when one boldly states that features are not homologous one is in essence rejecting the null hypothesis.

In statistics and in science there are two kinds of errors. A type 1 error occurs when one accepts a hypothesis that is not adequately supported. Acceptance of the null hypothesis in the absence of sufficient evidence constitutes a type 2 error. Here we discuss the ramifications of type 2 errors that result from default acceptance of the null when data are not sufficient to support the hypothesis. For example, you may have 90% confidence in the hypothesis you are testing, insufficient to accept the hypothesis based on the scientific norm of 95% confidence. Nonetheless, the probabilities suggest that the hypothesis is more likely to be correct than the null. Perhaps with a larger sample size the extra information would permit acceptance of the hypothesis and rejection of the null. In such circumstances the null should not be strongly embraced.

This consideration of types of statistical error suggests that extreme care should be taken not to blankly infer a lack of homology or shared ancestry simply because we can not muster sufficient evidence to strongly document a particular instance of shared ancestry in the system. Another way to put this is to say that absence of evidence is not necessarily evidence of absence. This is especially the case in an evolutionary discipline where data are missing, due to sampling or due to extinction of informative groups, and where it is difficult to define the precise elements that share ancestry. There may often be whole families of subtly different hypotheses that merit consideration (Chamberlain, 1890).

In evolution and development, sequence similarity of a given molecule often provides prima facie evidence for homology of the genes and their protein products. In such instances it is not clear that absence of homology is even the appropriate null model. Rather the questions that arise include: a) how “far one can go” in inferring shared ancestry in functions as well as sequence, b) which details or components of molecular function, developmental mechanism or morphology share ancestry, and c) which evolutionary scenario best explains the diverse aspects of the available data.

Despite the evidence of evolutionary relationship provided by sequence similarity, issues of homology have proved profoundly problematic in organisms that have evolved substantially in body plan or life history. For example, HOX genes are acknowledged to pattern the axes of invertebrate and vertebrate bilaterians alike, and based on the roughly parallel order in which the genes are expressed, few would now doubt that the A/P axes themselves are homologous in some aspect. Tetrapod limbs are similar axial structures that express HOX genes with a comparable ordering. Most of us would nevertheless hesitate before calling these limbs homologues of the A/P axis in flies. We might hesitate because vertebrate phylogeny informs us that the tetrapod limb is a novel feature exclusive to the vertebrates—thus we know these structures were not present in the shared ancestor. On the other hand, one could argue that these borrowed features of the vertebrate limb are serial homologues that share ancestry by proxy through the A/P axial structures. However, only a subset of the HOX genes used in A/P axis patterning are used in tetrapod limb development. Thus we might have to parse the argument more finely—rather than discussing the whole axis, perhaps we might wish to identify subsets of features that we
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We have a fair idea of the evolutionary origin of tetrapod limbs. Nevertheless, subtle differences in definition of the problem yield different answers. Such precision of argument is yet more critical where body-plan evolution is less well understood, as is the case for most invertebrate phyla.

Turning again to the type 2 error analogy, in the case of both engrailed and especially the Distal-less genes, there was initially great hope that these would be simple markers that clearly mapped features that everyone would agree were homologous. Clearly this is not the case and the frustration of many workers has been palpable. There has been a tendency to be dismissive and in the case of engrailed to say that the gene “just makes boundaries,” or that the Distal-less genes just makes things that “stick out.” Such statements imply that co-option and convergence are rampant and that the expression of these genes carries no historical information. Some feel that where genes appear so plastic in their expression they should be excluded from the analysis. However, we argue hypotheses must be finely parsed before any test of homology can be clearly performed and that without such a refined hypothesis of the evolutionary relationship between morphologic features, shared ancestry or its absence will be difficult to document. Consequently it may often be difficult to assess whether apparent evolutionary plasticity is real, or represents a failure to adequately refine or consider the appropriate set of hypotheses of homology. Secondly, even when data supporting homology are not forthcoming, it is a still more difficult exercise to refute any and all aspects of shared ancestry between two features in distinctly different organisms which have features in common, such as shared gene expression. This acknowledgement of the potential for type 2 errors of course is not an excuse to assume homology without substantiation, or to overstate the support for homology. This would constitute the more traditional type 1 error. Our major motivation is to continue to entertain a broad range of information and hypotheses rather than assume a lack of homology and discard hypotheses when they have yet to be adequately investigated.

Let us consider the work on the Distal-less gene, required for the formation of the distal portions of the Drosophila leg. It is apparently involved in the outgrowth of limb elements as suggested by its expression in the tips of the multiple elements in the complex appendages of crustaceans. The expression patterns of this gene in the limbs of onychophorans and polychaetes engendered considerable interest in that these patterns suggest common ancestry of the developmental program functioning in laterally borne limb/gill structures (e.g., Panganiban et al., 1997). These results soon found their way into textbooks. Subsequently work on a brittle star showed expression of the gene in the tube feet (Lowe and Wray, 1997), and many found it difficult to equate the tube feet of echinoderms with the parapodia of polychaetes or the arthropod limb. Nevertheless, tube feet are borne laterally along an axial structure—the ambulacrum—and they have locomotory and respiratory functions similar to protosome lateral appendages. Thus it can be construed that position and function criteria traditionally used to identify homology have been met.

There are multiple views on the relationship of echinoderm ambulacrum to axial structures in other Bilateria. Many workers infer that the ancestral echinoderm was a singly ambulacrate form where the ambulacrum was roughly coaxial with an overall body axis such as is the case in Cambrian stylophorans (e.g., Ubags, 1975). Such a perspective provides a basis for relating the ambulacra with the A/P axis in other Bilateria. This is not the only view and recent data suggest that HOX genes may be expressed along the oral/aboral axis in urchins (Arenas-Mena et al., 2000). However, in urchins the ambulacra parallel the oral/aboral axis, making it difficult confirm or refute a relationship between any of these axial structures and HOX genes.

Given the above it should be apparent that assignment of shared ancestry or homology is difficult when one is comparing distantly related taxa. It also seems doubly difficult to deny any evolutionary relationship—to discard homology when there is expression of sequence similar genes in similar settings in disparate taxa. We are confronted with the difficult task of reconstructing specific evolutionary scenarios between disparate organisms and we are at risk of committing an error both when we infer shared ancestry of a gene, developmental function or morphologic feature, or when we reject such ancestry.

**Conclusions**

We have examined three disparate issues that if not adequately considered will tend to limit the suite of hypotheses under investigation in evolution and development. In reviewing what we have said it may appear that we have a rather bleak opinion of the promise of the evo-devo field. Nothing could be further from the truth, as we see it a large suite of intriguing possibilities are arrayed before us. The acquisition of data continues to provide new illumination for the hypotheses in hand, and to reveal additional hypotheses for consideration. Thus we advocate the recovery of more developmental and developmental genetic data from a far wider array of taxa than have been investigated to date. Such an approach will undoubtedly produce exciting advances that continually reconfigure important aspects of our understanding of the evolution of animal development.

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