Developmental genetics and homology: a hierarchical approach

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New advances in developmental genetics are providing a bridge to connect the study of development and evolution. The successful integration of these fields, however, is dependent on having a clear understanding of the concept of homology. Therefore, developmental genetic data must be placed within the context of the comparative method to provide insight into the evolutionary and developmental origins of traits. The comparative analysis of traits derived from several hierarchical levels (genes, gene expression patterns, embryonic origins and morphology) can potentially reveal scenarios of developmental integration, opportunity and constraint. Moreover, this approach has implications for resolving modern controversies surrounding the concept of homology.

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Recent advances in developmental genetics have led to the fascinating discovery that the molecular mechanisms controlling embryonic development are universally conserved across all metazoa[1]. It was the initial characterization of regulatory genes controlling the development of body form in *Drosophila*[2] that prompted a search for their counterparts in other taxonomic groups[3,4]. Since this initial search, many comparative studies have revealed that these regulatory genes are highly conserved through evolutionary time, and may play a key role in the evolution of metazoan body plans[5]. Results emerging from this field have not only advanced our understanding of the gap between genotype and phenotype, but have also provided a new bridge to study the connection between development and evolution[6,7].

The successful integration of these fields via developmental genetics, however, depends on articulation of a clear understanding of homology, one of the most crucial and controversial concepts in comparative biology. The concept of homology ultimately guides the observations, interpretations and conclusions drawn from any cross-taxonomic comparison of the roles of regulatory genes. Thus, to use developmental genetic data in order to understand the evolutionary and developmental origins of traits, the relationship between this class of data and the concept of homology must be established[8–10].

Homology: modern concepts and controversies

Among the modern concepts of homology, the two most prevalent to emerge are the historical and biological[11] (see Ref. 12 for review). The historical homology concept places importance on phylogeny and evolutionary history as a primary guide in determining and explaining homology (e.g. Refs 13–16) (Table 1). Conversely, the biological homology concept defines homology on a mechanistic basis (i.e. developmental mechanisms) independent of phylogeny[17–20] (Table 1).

The controversies arising between these two concepts can be attributed to the fact that they implicitly emphasize different aspects of 'sameness'[21]:

> 'With growing knowledge the idea of sameness became more refined by the realization that there are various aspects to it: the same structural organization ..., the same developmental origin ..., the same developmental constraints ..., the same (genetic) information ... Most difficulties with the homology concept are because these various aspects of sameness are not congruent ... There are organs with the same structural organization but radically different developmental pathways; there are structurally identical body parts which use different genetic information for their development .... and structurally identical organs do not need to have a common phylogenetic origin' (Ref. 21, p. 274).

However, these conflicting aspects of 'sameness' should not be viewed as problematic, rather, they should be viewed as revealing the context dependence and hierarchical nature of homology. The homology concept can become more inclusive, and can potentially accommodate both historical and biological perspectives when rooted in a hierarchical approach, which proposes that the above 'aspects of sameness' be analysed simultaneously within a phylogenetic framework[16].

Developmental genetics and a hierarchical approach to homology

Examining homology as a property that is expressed at several levels of biological organization (i.e. genes, gene expression patterns, embryonic origins, and morphological structures) has recently received much attention[8–10,16,18,22–24]. The recognition that homology can exist independently at each of these levels has allowed the integration of developmental genetic data into a meaningful framework for the analysis of homology[8–10,24]. However, this class of data must be placed within the context of the comparative method[6,7,21,25,26], as it provides an explicit methodology to determine homology at several hierarchical levels of biological organization[16].

Using the comparative method, the homology of a trait can be recognized *a posteriori* based on a phylogenetic analysis incorporating many other characters (excluding the characters that are being tested)[14,16]. The first step in identifying a homologous trait at each level of the biological hierarchy is to formulate a

Table 1. Modern concepts of homology

Homology concept	Definition	Cause	Scientific goal
Historical	'Attributes of two organisms are homologous when they are derived from an equivalent characteristic of the common ancestor' (Ref. 13, p. 465).	Phylogenetic descent	To explain evolutionary origins and taxonomic distribution of traits through systematics[14].
Biological	'Structures from two individuals are homologous if they share a set of developmental constraints, caused by locally acting self-regulatory mechanisms of organ differentiation. These structures are developmentally individualized parts of the phenotype' (Ref. 19, p. 62).	Developmental mechanisms	To explain patterns in the mechanistic origin and evolution of morphological characters[21].

0169-5347/97/$17.00 PII: S0169-5347(97)01125-7

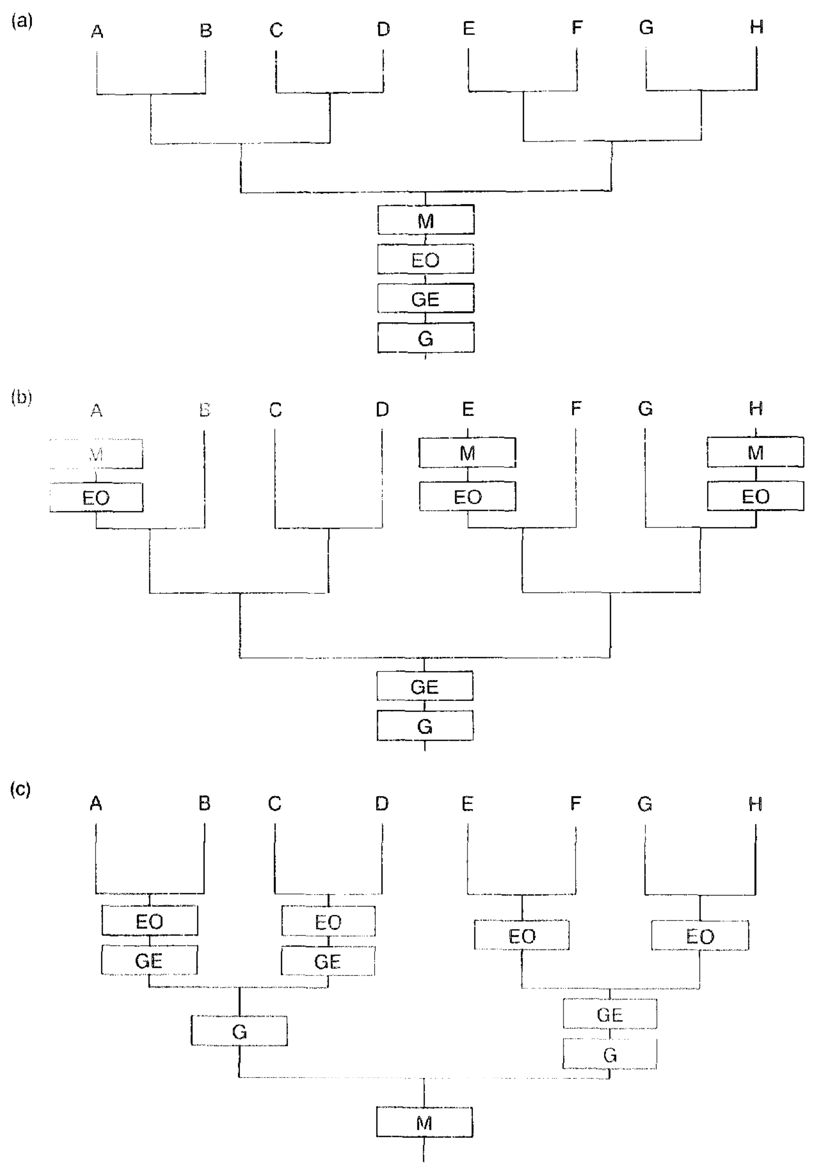

Fig. 1. Three hypothetical scenarios. The boxes represent traits derived from several levels of the biological hierarchy; gene structures (G), gene expression patterns (GE), embryonic origins (EO), and the morphological structure (M). These hierarchical traits are mapped onto a hypothetical eight taxon phylogenetic tree (taxa labelled A–H). (a) represents a scenario of developmental integration of the mapped characters, while (b) represents a scenario of developmental opportunity; (c) represents a scenario of natural selection maintaining the morphological trait in spite of the fixation of new developmental and genetic bases, or of morphostatic developmental constraints.

hypothesis of homology based on similarity criteria (e.g. the structural detail and position of morphological features)[27,28]. The traits of interest are then mapped onto a phylogenetic tree. Consequently, the hierarchical traits will either covary with the phylogeny, supporting the natural or monophyletic clades defined within the tree, or they will not, in which case the homology hypothesis will be falsified.

The power of this approach depends on the robustness of the phylogenetic hypothesis of the taxon in question, the validity of the assumptions of phylogenetic reconstruction, and the number of informative characters used to construct the tree[25,29]. Moreover its success also depends upon resolving traits from each of the proposed hierarchical levels, which in some circumstances may prove to be problematic given the interactive nature of the developmental process.

This method can provide insights regarding the proximate (developmental and genetic) and ultimate (evolutionary) causes underlying homologous structures. This approach is demonstrated in Fig. 1, in which three (of many possible) hypothetical scenarios are presented[10]. Comparative data from four distinct biological levels – genes, regulatory gene expression patterns, embryonic origins and morphology – are mapped onto a hypothetical phylogeny for eight taxa (labelled A–H). Although these scenarios may seem simplistic at first, they serve as an initial template for interpreting the more complex situations found in nature.

In the first scenario (Fig. 1a), homology occurs at all four levels of biological organization in all taxa. This correspondence between genotype and phenotype reflects a deep evolutionary conservation and integration of the phenotypic, developmental, and genetic features of the trait. Integration of these hierarchical traits imposes a constraint on their evolution, but at the same time it makes the evolution of complex systems possible. In other words, if key regulatory genes act as genetic switches, then modification of these switches via mutation may cause development to proceed in a different, but harmonious channel. As a consequence, coordinated changes in the phenotype become possible[28,30].

This may explain the phenomenon of transformational homology, in which homologous morphological structures have become either transformed or elaborated in particular evolutionary lineages to perform different or more complex functions. A promising example of this scenario may come from developmental and genetic investigations of the transformation from fish fins to tetrapod limbs[31]. Homologous regulatory genes important in both fin and limb morphogenesis, such as *Msx*, *Distal-less*, *Hox* and *Shh* are being studied in a comparative context in the hopes of understanding the mechanisms responsible for this important evolutionary transformation.

The second scenario (Fig. 1b) reveals that the gene structure and regulatory gene expression patterns are homologous in all taxa. The morphological structure and embryonic origins, however, have three independent evolutionary origins (lineages A, E and F). This scenario is interesting, as non-homologous morphological structures are being controlled by an ancient and homologous set of regulatory genes and their corresponding developmental pathway. This scenario, which has traditionally been called parallel evolution[30], represents 'developmental opportunity' in that homologous regulatory developmental genes can be potentially co-opted to function in the origin of new traits through evolutionary time.

An empirical example of this scenario comes from a recent study on the origin and evolution of animal appendages[32]. The authors compared and mapped the molecular mechanisms underlying appendage formation across six animal phyla, and examined the expression patterns of the homeobox gene *Distal-less* (*Dll*) and its vertebrate homologue *Dlx*. The results of this study reveal a situation of developmental opportunity (Fig. 1b), in which a homologous regulatory gene (i.e. *Dll/Dlx*) is expressed along the proximo-distal axes of non-homologous appendages; developing vertebrate limbs, polychaete annelid parapodia, onychophoran lobopodia, ascidian ampullae, and echinoderm tube feet. The expression of *Dll/Dlx* in these six coelomate phyla is most likely due to the independent co-option of *Dll/Dlx* several times in evolution.

The third scenario (Fig. 1c) is inversely related to the previous one, as the morphological structure is homologous in all taxonomic groups, but the other levels of biological organization are not. Thus, different developmental and genetic processes are controlling homologous morphological structures. These hierarchical patterns may imply a role for natural selection in maintaining the morphological trait, in spite of the fixation of new developmental and genetic bases. Alternatively, this scenario may be interpreted as evidence for the presence of 'morphostatic developmental constraints' (*sensu* Wagner[21]), which are limitations to phenotypic variation caused by segregation of the developmental mechanisms that generate and maintain phenotypic characters. This would allow for the evolution of interacting systems that tend to channel off the effects of single alleles, or alterations in developmental processes[21,33,34].

There are many documented cases of homologous morphological structures that possess variable development (see Refs 34,39 for review). The pattern formation of insect segments provides a good example, as the developmental timing, embryonic origins, and genes involved in segmentation among several insect orders are variable[36,37]. Differences in developmental timing and embryonic origins are reflected in the long-germ band (segments established after the blastoderm stage) versus short-germ band (segments established before the blastoderm stage) mode of insect development. These differences are also reflected at the molecular level, in which the developmental pair-rule gene, *even-skipped*, and the segment polarity gene, *engrailed*, play significantly different roles in patterning of the segments.

Implications

Incorporating developmental genetic data into the hierarchical approach can yield insight into the proximate and ultimate causes underlying the origins of morphological traits (Fig. 1), which implies that there may be no real distinction between the biological and historical homology concepts (Table 1). The biological homology concept can be subsumed into the historical at the level of developmental mechanisms. This implication, however, will become clearer as future studies are conducted at a variety of taxonomic levels to determine the frequencies and conditions under which each of the three scenarios in Fig. 1 occur.

Another important implication is that a trait representing one level of the biological hierarchy cannot serve as an infallible proxy for the determination of homology at other levels[35]. The homology of morphological structures, embryological traits, as well as developmental genes and pathways, must be determined individually through their mapping on a phylogenetic tree. This is justified, as Müller and Wagner[10] have recently shown that genes and developmental patterning mechanisms tend to evolve independently of morphological characters.

A recent debate: the evolution of eyes

Developmental genetic data, when viewed within a hierarchical framework, provide a powerful means of elucidating the concept of homology. This view, however, has yet to be widely accepted, resulting in controversy surrounding the role of developmental genetic data in assessing homology. The controversy primarily stems from the fact that many investigators now interpret shared patterns of regulatory gene expression as evidence of morphological homology (e.g. Refs 38–40). The recent debate on the evolution of eyes will elucidate the problems associated with ignoring the hierarchical approach in favour of this current trend.

A debate between Gehring[41] and Dickinson[42] on the homology of arthropod and vertebrate eyes was stimulated by the discovery that the *eyeless* gene of *Drosophila* is homologous to the *Pax-6* gene in mouse and the *Aniridia* gene in humans[43]. Each of these genes plays a similar functional role in eye morphogenesis, leading Gehring and his colleagues to conclude that 'because *Pax-6* is involved in the genetic control of eye morphogenesis in both mammals and insects, the traditional view that the vertebrate eye and the compound eye of insects evolved independently has to be reconsidered' (Ref. 50, p. 785). Dickinson[42] rejected this conclusion, and argued that the roles of these genes in eye development should be termed homologous only if other evidence suggests that an orthologous antecedent of both *eyeless* and *Pax-6* functioned in the development of an eye in a common ancestor of arthropods and vertebrates. He concluded that the genes controlling eye development in these morphologically different structures may be homologous but the structures in which they are expressed are not.

More generally, there are three important points to consider before accepting the hypothesis that arthropod and vertebrate eyes are homologous. First, the phenomenon of genetic co-option provides the most plausible explanation to account for the observation that homologous regulatory genes, such as *Pax-6* and *eyeless*, are expressed in non-homologous morphological structures[5,9,18]. When studying the molecular evolution of regulatory genes, their biochemical and developmental function must be considered separately. The biochemical function of *Pax-6* and *eyeless* are as general transcription factors (which bind and activate downstream genes), but their developmental function is their specific involvement in eye morphogenesis. The key point is that the biochemical function of these genes is highly conserved through evolutionary time, while their developmental function is relatively free to vary. This inherent property of developmental regulatory genes allows them to be independently co-opted to function in structures which clearly have independent evolutionary origins, and provides further caution against using shared patterns of regulatory gene expression as evidence of morphological homology.

Second, the orthology and ancestral functions of the *Pax* and *eyeless* genes must be determined before making any claims of homology between vertebrate and invertebrate eyes. Gene tree analyses must be used to establish the orthology (gene copies derived from speciation) of *Pax* and *eyeless* genes because sequence and functional similarity can often be misleading[44,45]. However, one must take into account the complication[8] that there are two copies of the *Drosophila* eyeless gene, *eyeless* and *twin of eyeless*[46], and nine copies of the vertebrate *Pax* genes (*Pax* 1–9)[47]. To infer the ancestral functions of these genes, the comparative method must be used to map their multi-functional roles across several taxa on a metazoan phylogeny.

Finally, there is a large body of morphological and embryological evidence supporting the independent evolutionary origins of arthropod and vertebrate eyes[48,49]. Taken together, these arguments support a scenario of developmental opportunity (Fig. 1b). Until a proper hierarchical analysis is performed on all the existing data, I agree with Dickinson[42] that there is no reason to reevaluate the traditional view that the vertebrate eye and the compound eye of insects evolved independently.

Conclusion

Wake emphasized the importance of the concept of homology by stating that 'Homology is the central concept for *all* of biology' (Ref. 50, p. 268). This vital, yet controversial, concept is the common ground for the fields of developmental genetics and evolutionary biology. Developmental genetic data, when analysed within a phylogenetic framework, are a powerful tool for understanding the hierarchical nature of homology, as well as the developmental and evolutionary origins of traits. The comparative analysis of traits derived from several levels of biological organization has the potential to reveal scenarios of developmental integration, opportunity and constraint. That this approach can reveal insights regarding the proximate and ultimate causes underlying morphological traits, suggests the possibility of resolving modern controversies surrounding the concept of homology. Furthermore, the inconclusiveness of using shared patterns of regulatory gene expression to determine morphological homology emphasizes the value of employing robust phylogenies to reconstruct patterns of evolution at different biological levels. These are exciting times as new advances in molecular and developmental biology are bridging the gap between genotype and phenotype. By integrating these new advances with a hierarchical concept of homology, we are providing a framework for the study of development and evolution.

Acknowledgements

I thank Rafael Zardoya, Axel Meyer, Jeff Levinton, Greg Wray, Mike Bell and Günter Wagner for stimulating discussion on this topic, and for their insightful comments on earlier drafts of this manuscript. I also thank my colleagues at the Max-Planck-Institute for Biophysical Chemistry, Goettingen, Germany; Urs Schmidt-Ott, Julia Forjanic, Marcos Gonzales-Gaitan, Felix Loosli, and three anonymous reviewers for improving the overall quality of the manuscript. This work was supported by a graduate fellowship from the Fonds pour la Formation de Chercheurs et l'Aide a la Recherche (FCAR) from the Quebec government.

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