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REVIEW

Comparative methods in developmental biology**

Michael K. Richardson[1,*], Jonathan E. Jeffery[1], M. I. Coates[2,3] and Olaf R. P. Bininda-Emonds[1]

[1] Institute of Evolutionary and Ecological Sciences, Leiden University, The Netherlands
[2] Biology Department, Darwin Building, University College London, UK
[3] Current address: Dept. Organismal Biology and Anatomy, The University of Chicago, USA

Summary

The need for a phylogenetic framework is becoming appreciated in many areas of biology. Such a framework has found limited use in developmental studies. Our current research program is therefore directed to applying comparative and phylogenetic methods to developmental data. In this paper, we examine the concepts underlying this work, discuss potential difficulties, and identify some solutions. While developmental biologists frequently make cross-species comparisons, they usually adopt a phenetic approach, whereby degrees of overall similarity in development are sought. Little emphasis is placed on reconstructing the evolutionary divergence in developmental characters. Indeed, developmental biologists have historically concentrated on apparently 'conserved' or 'universal' developmental mechanisms. Thus, there has been little need for phylogenetic methodologies which analyse specialised features shared only within a subset of species (i.e., synapomorphies). We discuss the potential value of such methodologies, and argue that difficulties in adapting them to developmental studies fall into three interlinked areas: One concerns the nature and definition of developmental characters. Another is the difficulty of identifying equivalent developmental stages in different species. Finally the phylogenetic non-independence of developmental characters presents real problems under some protocols. These problems are not resolved. However, it is clear that the application of phylogenetic methodology to developmental data is both necessary and fundamental to research into the relationship between evolution and development.

Key words: phylogeny reconstruction, comparative embryology, evolution, development

Introduction

Developmental biologists often make comparative studies. One example is the study of the evolution of developmental mechanisms. However, comparative datasets are not always concerned with evolution; developmental biologists may use them, for example, to assess the relevance of animal models to human development. Our aim here is to consider how developmental biologists analyse and present data in a comparative framework. This discussion is necessary because developmental biologists sometimes show an idiosyncratic approach to comparative studies, and rarely use the methodologies employed in other fields of biology. We are currently engaged in a research program to develop quantitative methodologies for comparative embryology, and so this paper is a statement of some of the conceptual issues underlying our work, and a discussion of the future directions that we see this work taking.

The need for a phylogenetic framework in examining biological phenomena is becoming increasingly appreciated in most areas of biology. However, such a framework has only a limited place in developmental biology. It has been used to map the evolution of developmental gene families (Ferrier et al., 2000) and to analyse relatively late events in ontogeny (Mabee et al.,

*Corresponding author: Prof. Dr. M.K. Richardson, Institute of Evolutionary and Ecological Sciences, Leiden University, Kaiserstraat 63, Postbox 9516, NL-2300 RA Leiden, The Netherlands; phone: ++31-71-527-5215; fax: ++31-71-527-4900;
e-mail: richardson@rulsfb.leidenuniv.nl
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2000; Velhagen, 1997). For the most part, however, developmental biologists use a type of comparative approach which phylogeneticists may not always recognise as being either legitimate or informative.
On the surface, this does not appear to have mattered – as the great advances made in understanding the molecular genetics of pattern formation in different species have demonstrated. There has been less success in integrating these data into a new synthesis of evolution and development. However, as interest in the diversification of developmental mechanisms grows (Burke et al., 1995; Tautz and Schmid, 1998; Wray, 2000), there will be a need for more analysis of early developmental characters (i.e., those expressed at stages when major patterning and morphogenetic events are taking place, and their interpretation under a phylogenetic framework). There are several reasons why phylogenetic approaches have not been widely adopted in developmental studies.
One is historical: phylogenetic methodologies were developed after embryology had lost its central place in evolutionary biology (Gould, 1977). When the debates about cladistics, and other methodologies, were taking place in the 1970's and early 1980's (Hull, 1980; Panchen, 1992), comparative embryology was seen principally as an auxiliary source, relative to outgroup comparison, of information about primitive conditions (e.g., in the debate about the ontogenetic criterion; see references in Meier, 1997).
Another reason is that developmental biologists are often most interested in conserved features, which are treated as if they were universal phenomena, largely because the vast majority of recent developmental research has been conducted within a purely experimental, rather than comparative, paradigm. Therefore, despite the clear phylogenetic baggage of a term such as 'conserved', interest in reconstructing the evolutionary history of changes in developmental mechanisms was minimal. Finally, the nature of development itself can raise severe obstacles to the adoption of quantitative methodologies.

Historical separation of comparative embryology from phylogenetics

Comparative methods used in modern developmental biology show several idiosyncratic and archaic features. These reflect its historical development. Embryology became isolated from zoology when it fell out of favour among evolutionary biologists in the first part of the twentieth century (Gould, 1977). The emphasis of developmental studies shifted instead towards experiments on developmental mechanisms. The discovery of organiser regions in the embryo focussed attention on apparently 'universal' developmental mechanisms or 'principles' that might operate in all metazoans.
Whereas classical embryologists often studied a wide range of species, chosen for what they might reveal about evolution (Richardson and Narraway, 1999), experimentalists relied on single, model species which could be easily reared for laboratory studies. Under these circumstances, 'universal' mechanisms were most interesting because they provided common ground for scientists studying different model animals. 'Universal' mechanisms also give legitimacy to the use of animal models in studying human disorders.

Universality or symplesiomorphy?

As noted above, developmental biology has its own terminology. Shared primitive developmental characters are described as 'conserved developmental pathways', 'developmental toolkits', 'deep homologies', 'Bauplans' etc. (discussed by Richardson et al., 1999; see also Coates, 1993). These terms do have special and useful meanings in their own right. But they can also serve to emphasise the phenetic nature of comparative developmental biology, and to downplay the importance of phylogeny. In some respects, this is acceptable, given that most studies in this field do not aim to reconstruct the history of changes in developmental mechanisms. However, without a phylogenetic methodology, we cannot hypothesise the phylogenetic level at which a character is 'universal' (i.e., 'shared'); nor can we test assumptions of primary homology *sensu* de Pinna (1991; i.e., whether the character evolved once, or convergently many times). Finally, we cannot determine whether absence is primitive or secondary.
For example, developmental biologists often assert that the apical ectodermal ridge (AER) is essential for outgrowth of paired appendages in 'all tetrapods' or even 'all vertebrates', even though an AER has long been known to be lacking in some amphibians (reviewed by Richardson et al., 1998a). In this example, a phylogenetic approach would provide information about whether the AER is a primitive character of gnathostomes, and whether its absence in amphibians is a derived condition.
The formulation of positional information theory (Wolpert, 1969) was a major impetus to the identification of universals. As noted by Richardson et al. (1999), Wolpert emphasised universal principles and mechanisms in development (Wolpert, 1969, 1989) and introduced the concept of a universal positional field in all animals. Wolpert's emphasis on universals was later adopted by scientists studying the molecular basis of pattern formation, and may have influenced such concepts of universality as the zootype (Slack et al., 1993) and developmental toolkit (Akam et al., 1994).

It could be argued that studies of 'universal' features have no need for a phylogenetic framework. However, we believe that the idea of universality carries with it tacit phylogenetic assumptions (i.e., the primary homology of the character being considered, and the relatedness of the species under study). It therefore requires the use of an explicit phylogenetic framework rather than the implicit (and occasionally naïve) evolutionary one that is sometimes used.

Difficulties with the analysis of developmental data

Comparative studies of development are, in principle, extremely complicated. They typically involve a 5-dimensional analysis. Morphology, or patterns of gene expression, are studied in 3 spatial dimensions within an embryo. Another dimension is the progress of development in one individual. Finally, the evolutionary dimension has to be considered by comparing development in different species. This complexity in the analysis makes it tedious, but not impossible.

However, more serious difficulties are raised by the nature of development itself. Unfortunately, it may prove impossible to solve all of these difficulties. The most difficult, and perhaps insurmountable problem, is trying to compare species at equivalent developmental stages. As discussed elsewhere, change in developmental timing (heterochrony) is one factor which may make this impossible (Richardson, 1995, 2001; Richardson et al., 1997). Thus, because of heterochrony, the landmarks use to define 'common stages' occur at different relatives times in different species.

And if it is true that there are no 'universal' developmental stages (such as those proposed by Witschi, 1956), then comparative embryology has no fundamental unit of comparison. And while this problem can be overcome by techniques such as event-pairing, these in turn can lead to further difficulties (e.g., character non-independence; see below).

Another problem lies in the definition of developmental characters. For example, as embryos pass through stages of morphogenesis and pattern formation, developmental characters undergo transformation and therefore disappear. And so, because developmental characters are not static, they need to be given defined start and end points (e.g., the otic pit is present *after* the otic placode has invaginated, but *before* the otocyst has detached from the ectoderm). There is also the issue of whether to score for the simple presence or absence of a character ('otic pit present'), or whether to score for a transformation leading to that character ('otic placode invaginating'). These issues are easily addressed by defining rules for character definitions.

Phenetic approaches

Phenetic approaches study overall similarities and differences without considering their historical relationships. von Baer's approach was essentially phenetic because he arranged animals into nested groups according to their degree of overall similarity (Fig. 1). This type of approach dominates comparative embryology, and is typified by the Haeckelian portrait-gallery diagram where species are arranged in columns, and developmental stages in rows (Fig. 2).

This type of diagram is widely used in textbooks, and has found favour as a teaching device. It provides a simple way to represent the 5-dimensional data of comparative embryology. Indeed it is difficult to think of a more succinct pictorial representation that would show different species and developmental stages simultaneously. Cladograms, for example, use single semaphoronts and cannot therefore accommodate different developmental stages.

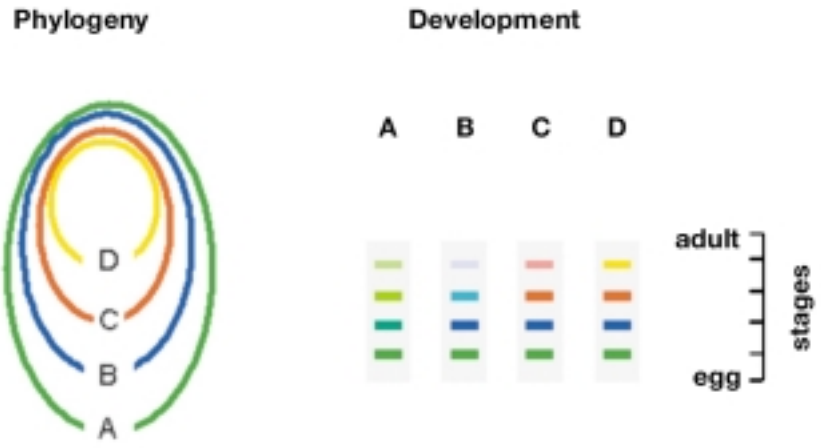

Fig. 1. Von Baerian phenetic approach to comparative embryology (4 imaginary species, A–D, are shown). The species are arranged in nested sets (left) according to similarity in their adult characters and not in a way that reflects evolutionary history. Note that there is modification of subterminal developmental characters (right) in this scheme. Thus all stages, except the very earliest, differ.

Nonetheless, the portrait-gallery diagram can be objected to on several grounds. First, the species are arranged so as to imply a linear transformation series reminiscent of the *Scala Naturae*; the true evolutionary relationships among the species depicted are thereby obscured. On similar grounds, Hanken (1993) has criticised the naïve evolutionary series implied in some developmental studies (e.g., where the zebrafish is said to be 'primitive' with respect to the chick; see also Coates, 1994, 1995; Metscher and Ahlberg, 1999).

Second, the reader is asked to believe that the horizontal rows represent embryos at comparable stages of development. As mentioned above, there are difficulties with this assumption, because heterochrony can alter the sequence of characters used to define the 'common' stages. Third, overall similarity is being compared, instead of characters. It is easy to see how the developmental hourglass (Raff, 1996) is derived from this type of portrait-gallery diagram (Fig. 2), and it is open to the same objections. Thus, both the portrait-gallery and the hourglass aim to convey gross phenotypic divergence, in a linear series of species, at different stages of development.

Despite these drawbacks, phenetic analyses have highlighted many interesting correlations, for example between developmental gene-expression patterns and adult morphologies. Thus, in one study, it was found that species with short necks showed a more cranial expression of the anterior *Hoxc-6* boundary than species with long necks (Burke et al., 1995). Importantly, this study provided key insights into similar processes and contrasting skeletal patterns, but did not concern itself primarily with the polarity of change.

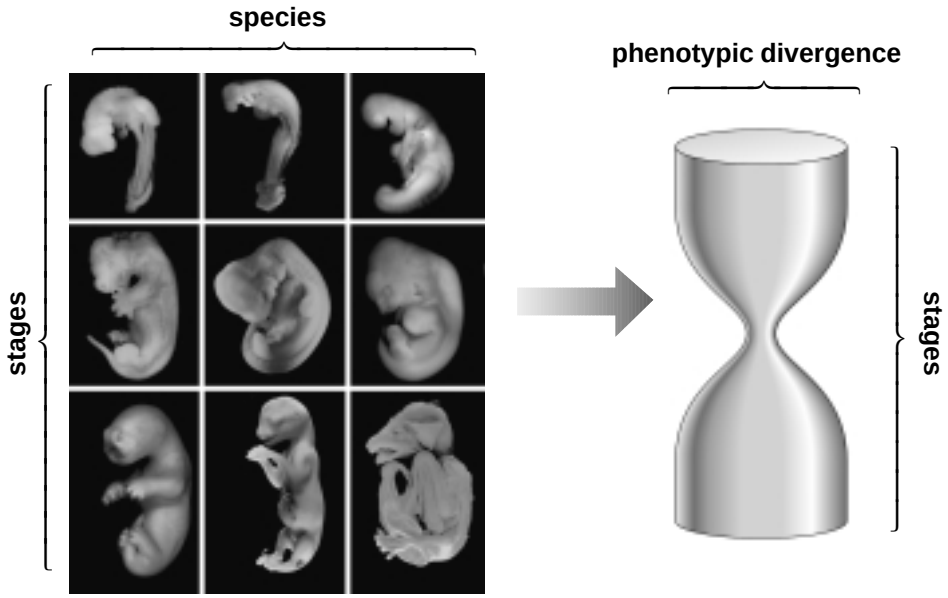

Fig. 2. The classic portrait-gallery of comparative embryology (left) and its derivative, the developmental hourglass (right). In this portrait gallery, the point of minimum phenotypic divergence is represented by the top row, whereas in the hourglass it is in the narrow, middle part. Both are stylistic conventions which often reflect an underlying phenetic approach. They do provide a simple representation of phenotypic divergence in development, though they can overlook the evolutionary history of the species studied, and imply, perhaps wrongly, that the stages examined are comparable. Figures on left from Richardson et al. (1998b); on right from Richardson (1999).

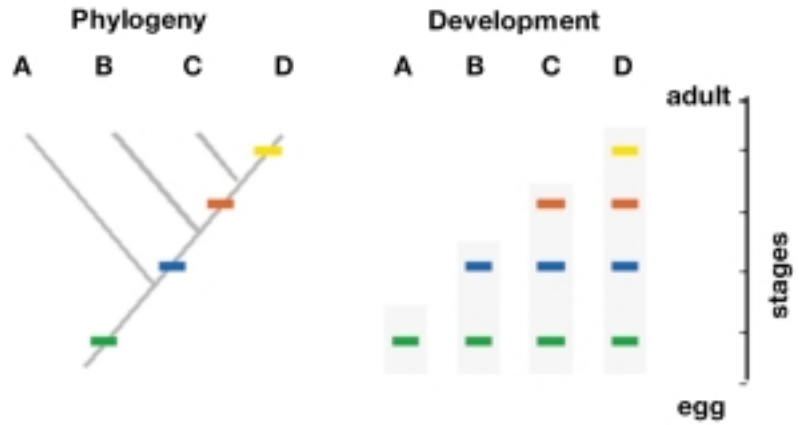

Fig. 3. The Biogenetic Law: evolution and development are treated as two parallel sequences. The model allowed for departures from a strictly parallel scheme (caenogenesis; not shown here).

Phylogenetic approaches

The Biogenetic Law

Ernst Haeckel was probably the first to systematically analyse development in an evolutionary context. Some aspects of his approach were phenetic, as shown by his use of portrait-gallery comparative embryology figures (Fig. 2). His Biogenetic Law formed the basis of his approach (Gould, 1977). We think it is important to discuss Haeckel's phylogenetic embryology because: (i) his methodology involved the analysis of developmental sequences to reveal information about ancestral conditions; (ii) he attached importance to heterochrony, believing that it obliterated the phylogenetic signal in developmental data. Developmental sequences and heterochrony are both important in modern phylogenetic methods (e.g., event-pairing), which are being used to examine developmental data in a phylogenetic context.

The important features of Haeckel's Biogenetic Law are embodied in his alphabetical analogy (Haeckel, 1896). This represents a phylogenetic series of species with the letters *A–Z*; the developmental sequence of a species in that series is represented by the same letters. Thus, the evolutionary appearance of a novel feature in a phylogenetic sequence is assumed to parallel its appearance in the developmental sequence (Fig. 3). But the sequence of stages, according to Haeckel, is not exactly parallel: thus there may be heterochrony (producing sequence changes such as *AB<u>DC</u>EF*); there may also be evolutionary transformations of stages or characters, such as *Aβ<u></u>CDEF*.

Interestingly, Haeckel believed that the principle role of development was to provide conserved characters for phylogeny reconstruction; he regarded specialised developmental characters as useless for this purpose because he believed that they 'falsify' the implied evolutionary history in the developmental sequence (Haeckel, 1896).

New approaches

Recently, advances have been made in the analysis of developmental data within a phylogenetic context. Developmental sequences are analysed to reveal sequence changes (heterochrony). One methodology is event-paring (Smith, 1996), which is used to provide an explicitly relative time frame in which to study heterochronic shifts (Mabee and Trendler, 1996; Velhagen, 1997). A pair of developmental events may have one of three timing relationships: Event *A* may occur before event *B*; events *A* & *B* may occur simultaneously; or event *A* may occur after event *B*. Each possibility is coded as a discrete character state (i.e., 0, 1 or 2, respectively here).

Event-pairing involves recording the relative timing of every possible combination of two events in the developmental sequence. The resulting event-pair scores for each species can be plotted onto a phylogenetic tree. Evolutionary changes in the developmental sequence will be highlighted by changes in the event-pair scores. There are advantages and disadvantages to this method. The main advantage is that event-pairing, by encoding the entire developmental sequence in a relative fashion, eliminates the problems of comparing species at similar stages of development. The main disadvantage is that it is difficult to reconstruct likely evolutionary changes in the developmental sequence based purely on the changes in event-pair scores (Smith, 1996, 1997).

Also, because an event-pair score results from the interaction of two developmental events, there is a high degree of non-independence within the collated event-pair data for each species. Because all events are compared to one another, a larger timing shift will affect more event-pair scores than a smaller shift (Smith, 1997). This can lead to problems when using event-pair data to reconstruct phylogeny, rather than simply optimising them onto phylogenies established by other means. We are currently using computer modelling to investigate the utility of event-pairing.

The importance of this type of work is that it promises to give insights into the relationship between evolution and development. This relationship is one of the central themes in biology and has been the subject of active debate for over a century. There is some confusion and disagreement, however, because the data appear to be contradictory. Thus, although there are examples of developmental novelties which violate the Biogenetic Law, others are consistent with it (Mayr, 1994). We believe that a methodology for analysing changes in developmental sequences will help cast light on this fascinating and complex issue.

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