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New directions in comparative embryology and the nature of developmental characters

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Abstract—Does developmental anatomy have a future in the age of molecular biology and digital technologies? Specifically, will morphological characters continue to be used in comparative developmental biology, or will new types of character be defined? Traditionally, comparative embryology was a non-quantitative, 'portrait-gallery' science. Wilhelm His attempted to develop a character-based, more quantitative approach. Quantitative approaches to development have been also been suggested by Meinhardt and others. With the current availability of computing power and the growth of bioinformatics and phylogenetic methodology, quantitative methodologies are increasingly being applied to studies of embryonic development. Our aim in this article is to examine some of these approaches. In both anatomical and molecular studies, the parameters to be quantified are temporal and spatial. Temporal data are analysed by techniques, such as event pairing, that analyse developmental sequences. In this case, the characters are developmental events. Spatial information can be analysed using morphometrics, in combination with computer-assisted 3D reconstruction. In spatial analyses, anatomical parts may be used as the characters. A major challenge in the coming years is to develop techniques for analysing 3D patterns of developmental gene expression and to compare them between species or individuals. Such analyses have to be defined in relation to five dimensions: the 3 orthogonal spatial planes; time; and individuals. The difficulties of such analyses are complicated by problems of homology. Some possible solutions are suggested. For example, it may be possible to use voxels as characters, and to assign to them attributes according to gene expression domains. At first sight, it might seem that traditional morphological characters would no longer be required in comparative embryology. However, we believe that some kind of anatomical framework will always be needed in comparative biology. The interplay between classical morphological characters, gene expression patterns and computing methodologies will be an exciting area for future work.

Keywords: comparative embryology; computational biology; developmental biology; embryology; event-pairing; 3-dimensional imaging.

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INTRODUCTION

Comparative embryology traditionally relied on the type of non-quantitative, portrait gallery approach popularised by Ernst Haeckel (Richardson et al., 2001). With this approach, hypotheses about evolution and development need no supporting evidence, other than pictures of embryos, and are formulated in the style of 'Naturphilosophie'. Wilhelm His (Sr.) criticised aspects of Haeckel's approach, and advocated a quantitative character-based analysis (His, 1874; reviewed by Richardson and Keuck, 2002). His noted that embryos lack the adult characters normally studied by zoologists. For this reason, he suggested that special developmental characters, such as epithelial thickenings, should be studied.

His even proposed a quantitative approach to the analysis of development — although it hardly provided a workable methodology. This pioneering form of developmental morphometrics consisted in making paper drawings of embryos, cutting round the outlines of the different organs, then weighing the paper cut-outs to get an idea of the size of different body parts. The data were then compared between different species. Many later workers used morphometrics to quantify growth patterns and mechanisms in development (reviewed by Huxley, 1932; Thompson, 1961; Gould, 1977; Meinhardt, 1982). However, relatively few of these studies relate to early (embryonic) development.

Our aim in this article is to look at some of the more recent quantitative approaches to the analysis of development. In particular, we want to see how they may affect the use and definition of the characters studied in comparative developmental biology. The principal methodologies discussed are for analysing developmental time (or developmental sequence), spatial or 3D patterns (morphology or gene expression), and developmental data in a phylogenetic context (as in cross-species comparisons). To date, phylogenetic analyses have principally been applied to developmental timing data, and we will mention them under that heading.

TEMPORAL ANALYSES (COMPARISONS OF DEVELOPMENTAL SEQUENCES)

The temporal component of development can be analysed in several ways. One is to treat development as a series of 'developmental events', where an event is a discrete change in character state. The events analysed with quantitative methodologies have, to date, been morphological changes. In principle, though, it is also possible to treat the onset of gene expression in a particular cell population as a developmental event. A series of events, ranked in chronological order, is a 'developmental sequence' (e.g., Alberch, 1985; Richardson, 2001).

The key methodology for analysing two or more developmental sequences is 'event-pairing' (Mabee and Trendler, 1996; Smith, 1996; Smith, 1997; Nunn and Smith, 1998; Smith, 2001; Jeffery et al., 2002a). This technique employs the pairwise comparison of events in the sequence, with each pair being assigned a numerical 'event-pair score'. The score expresses whether the events are simultaneous, or

whether event A takes place earlier or later than event B. In this form of analysis, the two events form the character, and their timing relations are the character state. A modification of the technique, called 'event-pair cracking' allows key heterochronic shifts to be inferred (Jeffery et al., 2002a).

In the future, event-pairing may also allow phylogeny to be reconstructed on the basis of developmental sequences. The legitimacy of using developmental sequences in phylogeny reconstruction remains controversial because of issues relating to the non-independence of sequence data (Jeffery et al., 2002a), as well as questions about character homology and homoplasy. However, we have recently used embryonic timing data to reconstruct a phylogeny for a group of vertebrates (Jeffery et al., 2002b). The results showed some congruence with a reference tree, as well as some discrepancies. Thus, while there may be some kind of phylogenetic information in developmental sequence data, the signal may not provide a complete record of phylogeny.

Another application of event pairing could be to compare the timing of events in individuals of the same species, but under different conditions. Thus we are currently exploring the use of event-pairing to look for timing shifts in the cardiovascular system of teleost embryos raised under hypoxia. In this study, the two sequences compared are those derived from hypoxia-raised embryos, and normoxia-raised controls ('t Hoen, Kranenbarg, van Leeuwen, Richardson and Witte, unpubl. data). Some shifts were indeed found, indicating phenotypic plasticity. A future goal would be to use event-pairing to look for correlated shifts in the timing of gene expression.

SPATIAL ANALYSIS (COMPARISON OF 3-DIMENSIONAL PATTERNS)

Anatomical patterns can be reconstructed in three dimensions by analysing serial histological sections or by exploring 3D images from the confocal microscope. Indeed, the use of reconstruction and modelling in embryology has a long history, predating even the wax-plate technique (Hopwood, 2002). In our own recent study on development of the chick heart, we used a combination of computer reconstruction of serial sections, scanning electron microscopy, and observation of whole-mounts, to make a final artistic reconstruction or model (Fig. 1). This represents a descriptive, non-quantitative form of data presentation. Gene expression patterns can be reconstructed, using similar techniques, from embryos processed for in situ hybridisation.

One objective of 3D reconstruction is to store the pattern in a database. This could allow cross-species comparisons, or studies of co-expression of genes. Once such databases are in place, links to other developmental databases, as well as other biomolecular databases, can be made. Exhaustive data mining can then be applied to address developmental problems (Verbeek et al., 1999). Several important issues need to be considered in this field, including the problem of how to define

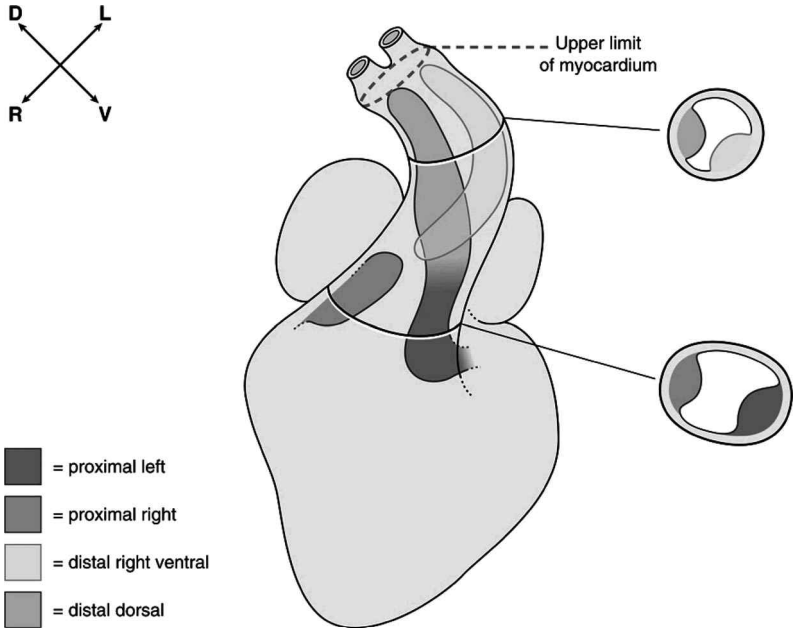

Figure 1. 3D reconstruction of chick embryo heart (from Quayyum et al., 2001). This kind of spatial information lacks a quantitative element. Cross-species comparisons using this kind of information would use named structures as the characters. However, homology could be a major problem.

3D matrices, the standardisation of data matrices between labs, and the homology between parts of the matrix or between datasets for different species.

The first problem in analysing 3D patterns is to devise some kind of spatial matrix, or set of coordinates, as a reference for defining the pattern. In morphological studies, the coordinates can be provided by anatomical landmarks (i.e., named structures or regions). This is the basis of traditional reconstructions of developmental anatomy whose output is essentially pictorial (Fig. 1).

Recent studies have developed a spatio-temporal reference matrix that allows patterns to be compared across stages or species. The matrix is available in a quantitative configuration, so that true spatio-temporal mapping can be achieved. These matrices are under construction for the mouse and the zebrafish (Baldock et al., 1997). In these studies, standard histology is taken as the starting point to produce a set of 3D models of selected time-points in development. These projects rely on a standardised vocabulary of the developmental anatomy (Verbeek et al., 1999).

We summarise the approach as follows. Histological sections are rendered as digital micrographs. Anatomical regions or named parts are then graphically annotated onto the images. In the case of the zebrafish this has been accomplished using the contour of an anatomical domain (Verbeek and Huijsmans, 1998; Verbeek, 2000; Verbeek et al., 2002). The precision of the mapping depends on the resolution of the micrographs. The result is a set of models whose regions are described by an anatomical vocabulary. The vocabularies themselves must be carefully defined if

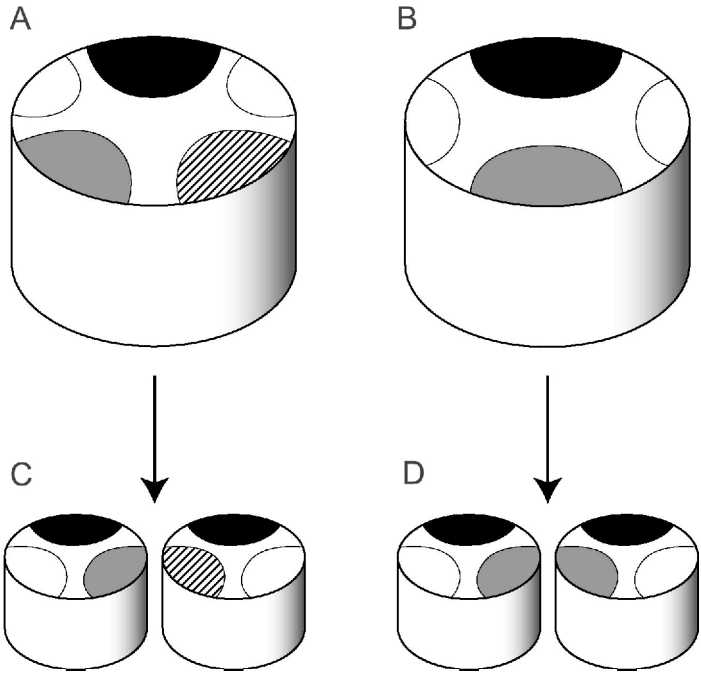

Figure 2. A typical developmental homology problem which may raise problems for databases that use anatomical name lists. A, B: schematic transverse cut-away section of the cardiac outflow tract of chick (A) and human (B) embryos. Note that there are two ventral cushions in the chick (grey and hatched in A) but only one in the human (grey in B). C and D correspond to A and B, respectively, after outflow tract septation and valve formation are complete. Note that the grey and hatched semilunar valves in C are derived from differently-named anatomical structures from those in D. Thus the anatomical names in A and B are not the same, and the homologies in C and D are unclear. Sources are reviewed in Quayyum et al. (2001).

they are to be comparable between studies. In such comparisons, the anatomical regions might be the characters, and the genes expressed in them would be the character states.

The models and vocabularies only make sense if they can be properly searched and examined in databases — a task that is dealt with in developmental bioinformatics. A typical example is the zebrafish atlas (Verbeek et al., 2002). Special attention is given to the spatial resolution; the histology model can be examined up to the cellular level and if required more detail can be added (Verbeek and Boon, 2002).

The problem of developmental homologies in the comparison of spatial patterns

To analyse patterns of developmental gene expression, it may be possible to simply list the organ primordia in which particular genes are expressed (i.e., map the gene expression onto a model whose regions are defined according to an anatomical vocabulary). However this approach raises several issues relating to homology (for an example, see Fig. 2). First, the landmarks or coordinates of the spatial

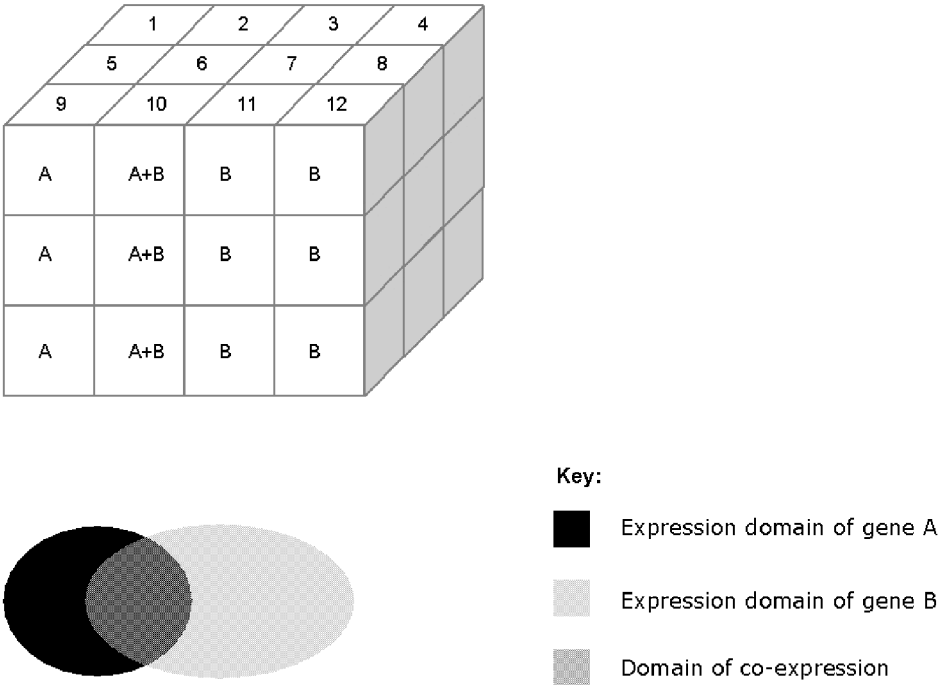

Figure 3. A voxel-based approach to comparison of spatial patterns of gene expression. At the top is shown a 3D array of voxels, each of which has a unique tag (the numbers on the upper face, for example). Each voxel represents a character which can be used in a comparative analysis. The character state of each voxel is derived, in this example, from the schematic gene expression pattern shown below. Each voxel gets a character-state tag that represents which gene or genes are expressed in the region that it overlaps.

matrix or model must be homologous between species (i.e., show taxic homology), and they must be homologous within the species (i.e., show serial homology). Second, anatomical landmarks can change their names and relative positions as development proceeds; they may even be transformed into structures with different anatomical names. In this respect, therefore, anatomical coordinates may not be stable across developmental time. A related issue is that of cell lineage. In the vertebrates at least, many anatomical structures in embryos do not behave as fixed lineage compartments; cells from other regions may migrate into a structure, so that it changes its cell composition, yet retains the same anatomical name.

Can we ever abandon anatomical characters when analysing spatial patterns of gene expression?

An alternative approach to using vocabularies might be to remove the anatomical matrix altogether. To do this, we suggest that the embryo could be treated as a series of 3D domains or voxels (a voxel or volume element is the 3D equivalent of a pixel or picture element, and is the smallest rectangular element into which

a 3D image can be broken down). Each voxel would then be characterised by a list of genes expressed within its boundaries. Finally, the spatial relationships of each voxel would be expressed in terms of its spatial co-ordinates in the matrix of voxels. Analysis of the co-ordinate relationships between voxels might be done using a modified form of event pairing. We summarise some aspects of our model in Figure 3.

A voxel may represent a single cell, or a group of cells, depending on the resolution chosen. The boundaries of a gene expression domain are expressed, in this analysis, by analysing the spatial relationships of voxels which do, and do not, respectively, express a given gene. Overlapping domains are defined by voxels that express both genes under study (Fig. 3).

This system is purely relational, in the sense that the only landmarks are the voxels themselves; relative position is defined by the position of one voxel in relation to others, as in a co-ordinate system with three axes. It would, of course, be possible to map anatomical features onto the voxel matrix by giving each voxel a name tag that represents which structure or region it coincides with. However, the voxel-based approach could, in principle, supply an array of gene expression domains defined purely in terms of their spatial relationships to one another.

One major problem with this approach is that the embryos in different species may be of rather different shapes, even though the same homologous structures are present. Thus there will be numerous mismatches in voxel position when attempts are made to map spatial data from a zebrafish embryo directly onto, say, *Xenopus.* However, we predict that certain relationships will always be constant (e.g., the eyes will always lie cranial to the otocysts, and will always lie lateral to the neural tube), and so on.

So, can anatomy be eliminated from the process altogether? Probably not, because even the voxel approach may require anatomical references to define the pattern when the data are first acquired. Thus, when the processed embryo is placed under the microscope, the observer is necessarily recording that a gene is expressed, for instance, in the eye, or the somites, and is using anatomical planes and terms to orientate the embryo under the microscope. Ultimately, it may turn out that there is no getting away from anatomy, even in the age of digital information and molecular biology. In any case, it is clear that anatomy provides a frame of reference for other data types. The interplay between classical anatomy and molecular biology will emerge as an exciting new field that interacts with techniques from computer sciences.

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