

The zootype and the phylotypic stage

J. M. W. Slack, P. W. H. Holland and C. F. Graham

What is it that defines an animal? The definition provided here, made on the basis of developmental biology, suggests methods for resolving phylogenetic problems.

WHAT is an animal? According to elementary textbooks of biology it is an organism that feeds, moves and responds to stimuli. But this definition is purely behavioural. Since the time of Geoffroy St Hilaire, there has been no morphological concept of what an animal really is. We propose that an animal is an organism that displays a particular spatial pattern of gene expression, and we define this pattern as the zootype (see Fig. 1). The zootype is expressed most clearly at a particular stage of embryonic development: the phylotypic stage for each individual taxon (Fig. 1 periphery). Hence the phylotypic stage is of critical importance not only for defining individual body plans, but also for relating these body plans across the whole animal kingdom. We believe that application of the concepts of zootype and phylotypic stage will establish a new programme of research in animal phylogeny.

The zootype

Recent work in developmental biology has shown that there is a class of gene that specifies relative position within the body. Although such genes are an essential link in the causal chain of spatial patterning, the boundaries of expression need not correspond to the boundaries of structures formed later, and the same combination of states of gene activity may lead to the formation of different structures in different organisms. Mutations within these genes, in the fruit fly *Drosophila*, transform defined regions of the body into the character of an adjacent region. Homologous genes exist in vertebrates, and where it has been possible to mutate them, shifts in morphological character have also been produced (reviewed in ref. 1).

The Hox cluster genes are the best known of these. They were originally discovered in *Drosophila*^{2,3} (Figs 2, 3), and are a subset of a wider class of genes that occur in all eukaryotes and contain a sequence motif called the homeobox (reviewed in refs 4, 5). This is a DNA-binding domain, and all homeobox-containing genes are thought to be transcription factors that control the activity of other genes. The discovery of the first Hox cluster type homeobox-containing gene outside *Drosophila*⁶ caused great excitement, and it was quickly proposed that the homeobox would become a 'Rosetta stone' for the study of animal

development, enabling us to read the epigenetic code of other animals on the basis of our understanding of *Drosophila*⁷.

By 1989 it had been found that there existed a similarity of spatial order of expression of Hox cluster homologues between embryos of *Drosophila* and mouse^{8,9}. The comparisons between insects and vertebrates have been extended and are now very well described and analysed^{1,10-12}. We know that there are four homologous clusters of Hox genes in vertebrates. Expression of the genes at the 3' end of each cluster commences at anterior body levels of the embryo, and for each gene moving along the chromosome in a 5' direction, expression commences at a more posterior level. In *Drosophila* there is a single, albeit split, gene cluster comprising the Antennapedia and Bithorax complexes, which shows clear homology to the vertebrate clusters. The relationship between gene position within the cluster and relative anterior expression limits in the embryo is the same as in vertebrates. The nomenclature of vertebrate Hox cluster genes has now been revised such that the genes within each cluster are numbered according to the position of the gene in the cluster¹³.

Although the Hox cluster genes are the best known, there are other examples of conservation of expression pattern of position-coding genes. Recently the vertebrate homologues of the *Drosophila* anterior genes *orthodenticle* and *empty spiracles* have been shown to be expressed in the head of the mouse embryo¹⁴. This extends the similarity of anteroposterior pattern into the head, often thought of as an evolutionary novelty in the vertebrates¹⁵. There is also a similarity of the *even-skipped* expression region, which lies in the extreme posterior of the mouse, *Xenopus* and grasshopper embryos¹⁶. (In *Drosophila* the expression of *even-skipped*¹⁷ is complicated by its early pair-rule function, which may have no counterpart in distantly related organisms.) On the other hand, there are many other genes that we believe to be of developmental importance and that have homologues between animal phyla, but which do not show similarity of expression patterns between *Drosophila* and mouse, for example, *engrailed*, the *Pax* genes, *Pou* genes and *Wnt* genes.

This much is familiar to developmental biologists, but even they may not realize how widely spread the comparisons now are. It has been shown that

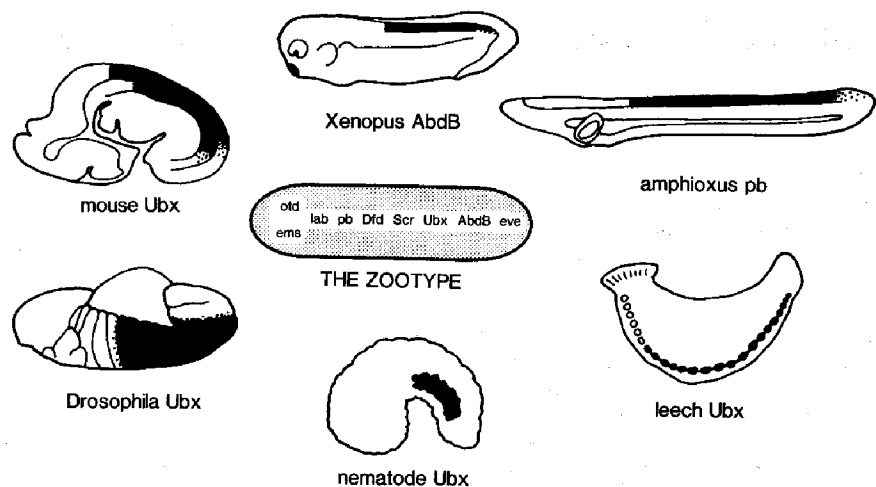


FIG. 1 The zootype (centre), showing the spatial order of anterior expression limits of the Hox cluster and some other genes. Around the zootype are shown phylotypic stages of various animals, showing the expression of individual genes as seen in whole mounts. *Drosophila* nomenclature is used for the genes: otd, *orthodenticle*; ems, *empty spiracles*; lab, *labial*; pb, *proboscipedia*; Dfd, *Deformed*; Scr, *Sex combs reduced*; Ubx, *Antennapedia-Ultrabithorax-abdominal A group*; AbdB, *Abdominal B*; eve, *even-skipped*. The specific nomenclature for animals and genes shown is as follows. Mouse, Hox 1.1 (=Hox A7); *Xenopus*, XIHbox 6; amphioxus, Amphihox 3; *Drosophila*, Ubx; *Caenorhabditis elegans*, mab-5; *Helobdella*, Lox-2.

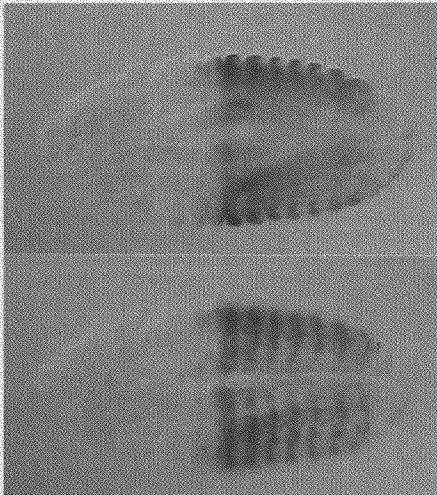


FIG. 2 Ubx protein visualized in the retracted germband stage of a *Drosophila* embryo by immunoperoxidase staining. Lower, ventral surface view; upper, optical section through deeper level. Anterior is to the left. Photographs courtesy of P. Ingham, ICRF Developmental Biology Unit.

genes of the Hox cluster have comparable expression patterns in amphioxus¹⁸, leech^{19,20} and nematode^{21,22}. Each of these carries a special significance. The amphioxus-vertebrate comparison suggests that the vertebrate head is homologous to the anterior, but not cephalized, segments of the lower chordate. The leech-insect comparison shows that the patterns persist even though the modes of segment formation are very different. The nematode results show that the same expression pattern can be present in an organism with an invariant cell lineage and a very small total cell number. In other words, the Hox cluster genes really do seem to encode relative position within the organism rather than any specific structure, and the patterns are conserved despite major shifts in other developmental mechanisms. Some people have proposed that Hox gene activities, or combinations of activities, do encode particular structures, for example the different types of vertebrae in mammals²³. It is likely that these associations will be found within a phylum but the inter-phyletic comparisons suggest that the basic function is to code for relative position. The existence of such 'positional values' was predicted some time ago by Wolpert on the basis of classical experimental embryology²⁴. He made the explicit prediction that the coding system should be universal, and this does now seem to have been borne out.

Hox cluster genes are also present in *Hydra* (phylum Cnidaria). Although the normal expression pattern here is not known, when the head is amputated and a new head regenerates from the stump, the labial homologue, *cnox-1*, comes on

before the *Dfd* homologue, *cnox-2* (ref. 25). In organisms capable of bipolar regeneration, the rule is that the extreme positional coding becomes established first and intermediate codings are then intercalated from anterior to posterior²⁶. So if *cnox-1* is turned on before *cnox-2* in regeneration, this suggests that *cnox-1* represents a more anterior coding than *cnox-2*, consistent with the expression of their homologues in other animals. The presence of Hox cluster genes has also been reported in the fluke *Schistosoma* (phylum Platyhelminthes), although no expression data is yet available²⁷.

Because it looks strongly as though a system of gene expression patterns, comprising the Hox cluster type genes and some others, do encode relative position in all animals, it is probable that this system is very ancient and was in place in the common ancestor of all modern animals (Fig. 4). We now suggest that this character should be adopted as the defining character, or synapomorphy, of the kingdom Animalia. We propose that it be called the zootype.

The phylotypic stage

It is widely appreciated that each animal phylum can be represented by a particular general body plan²⁸. It is less widely known that there is a stage of development at which the precursor to this body plan is actually visible. This was first defined as the stage of the *Körpergrundgestalt* by Seidel²⁹, and called the phylotypic stage by Sander³⁰. The phylotypic stage can be variously defined as the stage of development at which all major body parts are represented in their final positions as undifferentiated cell condensations, or the stage after the completion of the principal morphogenetic tissue movements, or the stage at which all members of the phylum show the maximum degree of similarity. The following seem to meet these criteria: the tailbud stage for the vertebrates; the fully segmented germband stage for insects; the fully segmented, ventrally closed stage for leeches; or the nematode after the completion of most embryonic cell divisions.

The phylotypic stage of development is not the earliest stage. Early stages can be quite variable even within phyla or classes. Some examples of such divergence are the arrangements of extraembryonic

membranes in different groups of mammals³¹, the modes of segmentation in short and long germ insects³², or the existence of holoblastic and meroblastic cleavage in different groups of fish³³. It is generally presumed that this variability of early stages results from adaptation to particular types of reproductive strategy or to the demands of embryonic nutrition. The conservative phylotypic stage lies between the variable early stage and the variable late stage at which the morphology of the postembryonic organism is formed in miniature, and is again susceptible to adaptive change.

It is a remarkable fact that, for each individual phylum so far examined, the embryonic stage at which the zootype is most clearly displayed is the phylotypic stage of the group concerned (see Fig. 1). The genes of the zootype are not, in general, activated in the earliest stages of development, and although expression may persist for some considerable time, the peak expression, and the simplest pattern of expression, is displayed at the phylotypic stage. This association gives us confidence that the independent proposals of evolutionary conservatism of the zootype and of the phylotypic stages are indeed well founded.

Implications of the zootype

From the existing data it seems that non-animal multicellular eukaryotes such as plants, fungi and slime moulds have homeobox-containing genes, but that these do not fall into the homologous families of the genes whose expression patterns comprise the zootype (T. R. Burglin, personal communication). There are also no similarities of expression between these organisms and animals. The definition of the zootype therefore gives us, for the first time this century, a morphological criterion for what an animal is. It is more precise than the behavioural criteria used hitherto, and has both theoretical and practical implications.

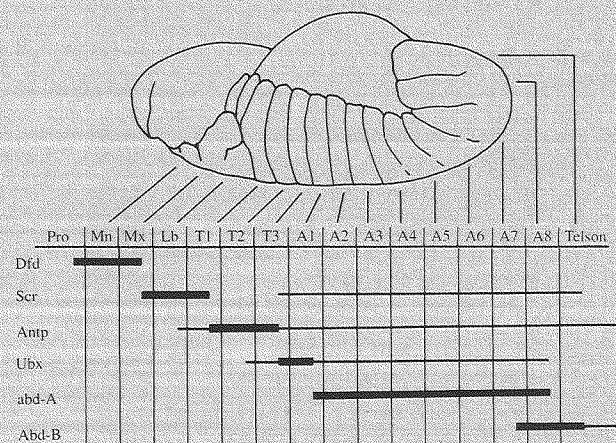


FIG. 3 Expression domains of Hox cluster genes in the *Drosophila* embryo.

First, we may consider the reason for the persistence of the zootype. The evidence from the effects of overexpression, or mutation to inactivity, of the genes of the zootype, is that their function is to encode different relative positions within the embryo. Their functions are informational rather than executive. Information units are arbitrary in that one could be substituted for another without affecting function, so it seems probable that the persistence of the zootype indicates common ancestry rather than convergent evolution driven by considerations of molecular efficiency. So we believe that there was once a primordial multicellular ancestor of all existing animals and this ancestor was the first organism to possess the zootype (Fig. 4). The zootype has persisted ever since, perhaps because, although it is arbitrary, mutational change to it would be too disruptive to the rest of the developmental programme of a plausible animal. We are unable to say anything about the visible morphology of the proto-animal because the zootype is a system of positional information and does not necessarily encode any particular structures.

Second, although the zootype is not visible on casual inspection, the presence of the genes can be established using the polymerase chain reaction on preserved specimens and, so long as a phylotypic stage is available, the expression patterns can be visualized on preserved specimens by *in situ* hybridization. It is therefore a practical taxonomic tool for deciding the affinities of various 'borderline' organisms. These would include the sponges (phylum Porifera), the Mesozoa, the Placozoa and various groups of Protozoa³⁴.

Third, although the definition of phy-

lotypic stage given above is clear enough for most groups, there are ambiguities. For example, most echinoderms have motile larvae. These come with various morphologies (pluteus, auricularia, vitellaria and so on) which do not entirely correspond to the class divisions defined by adult morphology³⁵. Is the phylotypic stage the larva or the metamorphosing adult? If we accept the evidence from the other phyla then the zootype must be displayed at the phylotypic stage, but need not be at other stages. Hence examination of the

zootype in echinoderms could tell us which stage is really phylotypic. This would tell us which stage should really be compared to other phyla in the establishment of super-phyletic groups such as the Deuterostomia. Evolutionary biologists may also be able to decide whether those echinoderms without larvae³⁶ are primitive or show secondary loss of the larva.

Could parts of the zootype itself be subject to secondary loss? The zootype has clearly persisted for a very long time in evolutionary history and this may suggest that the maintenance of a complex morphology needs the system of positional information which is provided by the zootype. However, it may be possible for highly degenerate creatures, such as parasites with little remaining morphology, to have lost parts of the zootype. To a strict cladist this possibility would mean that the zootype was not a good synapomorphy for Animalia. We

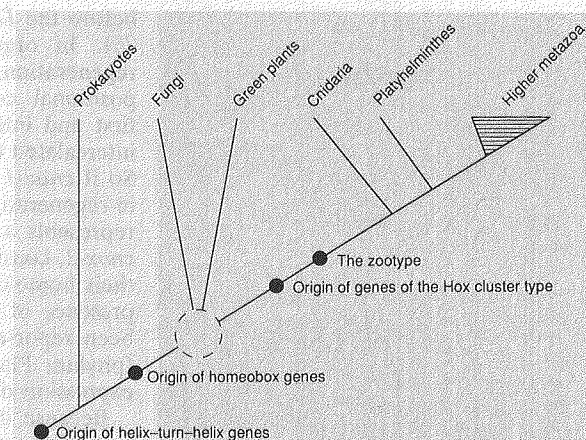


FIG. 4 Origin of the zootype on the evolutionary tree. The Hox cluster genes are a subset of the homeobox genes, which are in turn a subset of genes encoding DNA-binding proteins of the helix-turn-helix class.

would take the view that a plant which has lost its chloroplasts remains a plant because of the weight of other correlated characters, so perhaps the same attitude should be taken to the possibility of secondary loss of parts of the zootype.

Finally, the recognition of the zootype could provide a rational basis for large-scale animal taxonomy. The existing groupings above the level of the phylum are somewhat insecure, mainly because of the difficulty of attempting to find morphological homologies between different body plans³⁵. But if the zootype is common to all animals, then the next layer of genes controlled by the zootype codings is likely to represent the next fundamental level of body patterning. Once these genes have been identified and their functions understood, then they will make up the basic characters for defining phyla and super-phyla. This area of molecular developmental biology must now be rated as a particularly important one for those studying taxonomy.

To an extent the zootype represents a rehabilitation of the concept of the archetype of all animals, promoted by Geoffroy St Hilaire in the first part of the nineteenth century³⁷. Little could he have known that the zootype can best be observed at a stage of development that was just beginning to be described in those days, and that its observation would require techniques that would not be invented for nearly 200 years. But the zootype is not just a novel means of defining the animal kingdom; it also leads us to make a number of testable predictions about phylogenetic problems. We await the results of these tests with interest. □

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