The evolution of insects and vertebrates: homeobox genes and homology

The impressive increase in knowledge of molecular biology has led to much enthusiasm for its apparent predictive and explanatory power. In many respects this enthusiasm is justified. However, some of it lacks realism, in particular with respect to the implications for inferring homology among complex anatomical characters. For instance, several papers (e.g. Refs 1,2) have suggested homology of anatomical structures on the sole basis of similarity of homeobox genes, the genes that play a pivotal role in pattern formation during development. Yet the knowledge that, sometimes, hundreds of millions of years of evolution separate the taxa under consideration should warn us that, in spite of the tendency of homeobox genes to be highly conserved, there is a definite possibility that the relationship between these genes and morphological structures has been subject to extensive changes. A new article by Müller and Wagner[3] argues convincingly that it is unreasonable to infer the homologies of structures from genetic data without thorough consideration of the developmental pathways (see also Ref. 4).

First, it is important to assess the evidence that homologous homeobox genes are involved in the formation of homologous, or alternatively non-homologous structures in vertebrates, insects and animals in general. It turns out that it is easier to find good examples of homologous homeobox genes involved with non-homologous than homologous structures when comparing insects and vertebrates. An example of the former is provided by the *Brachyury* (*T*) gene in vertebrates and the homologous *T*-related gene in insects[2]. In vertebrates, the *Brachyury* (*T*) gene has a role in the development of the notochord, whereas in insects the *T*-related gene is involved in the formation of the hindgut. Even within the insects, examples can be found of homologous homeobox genes involved in the development of non-homologous structures. In *Drosophila*, the homeobox gene *distalless* is involved in the proximo–distal organization of adult limbs and wings[5]. In the much-studied butterfly *Precis coenia*, the *distalless* gene is expressed in the prolegs (larval locomotory appendages) and in addition in the area in the wings from which eyespots arise[5]. The prolegs may well be homologous with the adult legs, but the eyespots of the wings are not homologous with the wings. Examples where homologous genes are clearly involved in the formation of homologous structures and not merely specifying similar positional information (see below) have only been documented within insects or within vertebrates, that is, in more-related organisms[3].

Secondly, it is even more important to understand why homologous genes are not always involved in the establishment of homologous structures. There are several different reasons why this should be so:

(1) Two duplications of *Hox* gene clusters have probably occurred in the evolution of vertebrates, which supposedly has led to redundant genetic information[6]. Müller and Wagner[3] argue that this redundant genetic information was used differently in independently evolving vertebrate lineages, with the result that homologous *Hox* genes are involved in the development of non-homologous structures. Evidence for the independent evolution of duplicated *Hox* gene clusters comes from Misof *et al.*[7], who found that some genes from the duplicated *Hox* clusters were lost in the tetrapod lineage but not in the teleosts. The best example perhaps is the fact that there are four group 10 genes in zebrafish (Ref. 7; Prince, pers. commun.) but only three in mouse and man. This could be even a teleost characteristic since there is also evidence for four group 10 genes in the killifish[8].

A case in point for the differential use of duplicated homeobox genes are the individual *HOM* genes in the one cluster that insects have (e.g. Ref. 9). The HOM proteins that they encode are very similar to one another whereas their effects are diverse. They are presumably derived from a single ancestral protein. Yet these proteins have remarkably different functions in *Drosophila*[9].

(2) Some homeobox genes signal the same positional information in insects and vertebrates and thus have the same function – that is, telling cells that they are in a specific position – but the information is used to build different, non-homologous structures[3,4]. The evolutionary change in this case occurred somewhere downstream from the *Hox* genes. This illustrates the hierarchical nature of the homology concept. An intuitively easy example of such a hierarchy is mentioned by Dickinson[4]: the wings of birds and bats are homologous when considered as forelimbs, but they are definitely not when considered as wings. An example dealing with homeobox genes can be found in the very similar homeobox genes that are involved in the initiation of eye morphogenesis in *Drosophila*, mice and humans[1]. This function of the genes is homologous, but as Müller and Wagner[3] argue, the eye structures of insects and vertebrates are certainly not homologous (see below for further explanation).

(3) Pleiotropic homeobox genes may lose a function during evolution, where the function lost varies among different descendant species. Certainly, many *HOM/Hox* genes have pleiotropic effects, e.g. the abovementioned example of the *distalless* gene which is involved in the formation of both the prolegs and the eyespots in the butterfly *Precis*[5]. In addition, loss of function is likely to occur in homeobox genes[10].

(4) Development is an interactive process[3]. The function of *HOM/Hox* genes depends on the epigenetic context in which they are expressed. Consideration of the developmental pathway is, therefore, a *sine qua non* when looking at homology. After all, evolution does not proceed from adult character to adult character as it may seem when looking at phylogenetic trees, nor does it proceed from genes and adult characters to genes and adult characters. Evolutionary changes alter developmental pathways and changes in developmental interactions are essential for the causation of structural novelties[3]. It is a fact that the development of arthropods and insects is dramatically different in certain respects, especially in the behaviour of tissues[11,12].

Firstly, in arthropods, tissues are sheets of connected cells (epithelia) that develop relatively autonomously. Vertebrate development not only involves epithelia, but also meshworks of isolated cells (mesenchyme), which migrate and interact with locally present epithelia[11,13]. A good example are neural crest cells (which migrate from the neural ectoderm to develop in interaction with different types of epithelium into such diverse cell types as the neurons and supporting glial cells of most of the peripheral nervous system), the epinephrine-producing cells of the adrenal gland, the pigment cells of the skin and skeletal and connective tissue components of the head[11,13].

Secondly, development in vertebrates progresses via a series of interactions as a cascade of mutually inductive effects[11,13]. The development of the eye is a good example to illustrate the concept[11,13]. Typically, the optic vesicle, which is part of the forebrain, induces in the overlying ectoderm the formation of lens cells. The lens cells reciprocate and induce changes in the optic vesicle, which transforms into the optic cup which subsequently differentiates into iris and neural retina. The lens, in addition, induces the overlying

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ectoderm to transform into transparent cornea. It can be seen from this series of interactions which is completely absent in insects, that the eye structures of vertebrates cannot be homologous with those of insects.

There is, therefore, no single way in which evolutionary changes take place. Genetic mutations can have an effect at each level of the hierarchical organization of development, and novelties can occur due to mutation effects on each of these levels. Small mutational changes can have small effects, but also dramatic effects depending on the epigenetic cascade of interactions. This is the reason why it is problematic to compare structures of insects with those of vertebrates for homology and, worse, why the whole concept of homology is so difficult to interpret and consequently so controversial. Some even believe that we should not attempt to atomize traits and regard them as homologous or non-homologous (e.g. Ref. 14). This seems too pessimistic a view, for in practice, characters can often be distinguished well enough to allow comparison between organisms (e.g. Ref. 15). The development of complex organisms is organized such that there are many locally and functionally integrated units[15]. Nonetheless, the diverse nature of evolutionary changes constrains the unequivocal determination of homology. Depending on whether the focus is on evolutionary patterns or processes, people tend to emphasize different aspects of homology (e.g. same genes, same developmental pathway or same adult structures), even though process and pattern are, of course, not independent[11].

The bottom line is that genetic identity does not necessarily coincide with morphological identity. Gene expression patterns are an important new source of embryological information, but need to be seen in context. As Müller and Wagner[3] show, the relationship between genetic and morphological identity can only be understood after an analysis of the developmental pathways. Ironically, it then turns out that genetic similarity, just as morphological similarity, is in itself insufficient to demonstrate homology conclusively[3].

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