Evolutionary Developmental Biology (Evo-Devo)

The importance of development in evolution has long been recognized. For example, Charles Darwin devoted a chapter of *On the Origin of Species* to the subject of embryology. Darwin realized that evolutionary relationships of species that are morphologically very different as adults could be inferred from similarities in their early developmental stages, such as larvae. Similarly, he noted that the great diversity of adult morphological forms must arise through differences in development.

With the rise of molecular biology, it became possible to identify the genes that control developmental processes and to study them comparatively among species. This led to a resurgence in the field of evolutionary developmental biology, which has come to be known as "Evo-Devo".

Many of the early genetic discoveries of Evo-Devo came from studies of the fruit fly *Drosophila melanogaster* and were later extended to other organisms to reveal remarkable similarities in the developmental programs of diverse organisms.

1. *Hox* genes

Mutations that change one body part into another are known as homeotic mutations. In Drosophila, some examples are:

Ultrabithorax (*Ubx*) – mutations in this gene cause the third thoracic segment of the fly to develop like the second thoracic segment. This results in a fly with two pairs of wings instead of the normal one pair.

Antennapedia (*Antp*) – mutations in this gene can convert antennae into legs, resulting in a fly with legs growing out of its head.

These examples show that a single gene can have a major, controlling effect on the development of the adult body.

In Drosophila, there are eight homeotic genes that are located in two clusters within the genome. Five tandem genes form the *Antennapedia* complex (*Antp-C*) and three tandem genes form the *Bithorax* complex (*BX-C*). Together, these clusters of genes are known as *Hox* genes.

The *Hox* genes share a highly conserved region of 180 bp called the homeobox that encodes a 60-amino-acid domain of the protein known as the homeodomain. The homeodomain is a protein domain that binds to DNA, indicating that *Hox* genes encode transcription factors (proteins that regulate the expression of other genes).

Interestingly, the physical order of the *Hox* genes within their clusters corresponds to their anterior-posterior domains of expression in the developing Drosophila embryo. That is, the first gene has the most anterior expression (head) and the last gene has the most posterior expression (abdomen).

Hox genes are so well conserved that the Drosophila *Hox* genes could be used as probes to identify *Hox* genes in other species, including humans. It is now known that *Hox* genes are present in all animals. In humans and other vertebrates, many of the *Hox* genes are present in

four copies located in different clusters on four different chromosomes. This led to the hypothesis that the whole genome underwent two rounds of duplication during the evolution of vertebrates (known as the 2R hypothesis).

Not only are the sequences of *Hox* genes highly conserved, but also their functions and expression patterns. For example, the anterior-posterior pattern of expression of the *Hox* genes in the mouse embryo corresponds to the order of the genes along the chromosome – just like in Drosophila.

2. The *eyeless* gene

Fruit flies with a mutation in the *eyeless* gene fail to develop eyes. Note that in Drosophila, many genes are named after the phenotype that results from a mutation in the gene. Thus, the wild-type *eyeless* gene is necessary for the normal formation of eyes.

The vertebrate homolog of *eyeless* is known as *Pax6*. Mutations in *Pax6* lead to defects in eye development in both mice and humans. This suggests that the gene has a conserved function in eye development. Furthermore, when the Drosophila *eyeless* coding sequence is replaced by the human *Pax6* sequence, it can signal for normal eye development in the fly. If the *eyeless* or *Pax6* gene is mis-expressed in Drosophila, it can lead to the formation of eyes in the wrong place. For example, eyes growing on a fly's leg!

Traditionally, the eyes of Drosophila (compound eyes) and human (camera-type eyes) were thought to be analogous structures that evolved independently. Why do they use the same gene to signal development? Some possible explanations:

a) Eyes did not evolve independently, but were present in a common ancestor
b) The same gene was recruited independently for eye development in different lineages
c) An ancestral, very simple light-sensing organ used *eyeless*/*Pax6* and this gene was retained to signal the development of more complex eyes of different types

3. What about plants? MADS-box genes

In plants, there are homeotic genes that control floral development. They are also transcription factors, but instead of a homeobox sequence (like in the *Hox* genes), they contain a conserved sequence known as the MADS-box. The name comes from four different genes from different species that contain this domain (MCM1, AG, DEF, and SRF). The MADS-box is usually 180 bp (60 amino acids) and encodes a DNA-binding domain. In these respects it is very similar to the homeobox. However, the MADS-box and the homeobox do not share sequence homology.

Note that plants also have *Hox* genes and animals also have MADS-box genes. However, the relative importance of these two groups in development appears to differ between plants and animals.

4. The role of *cis*-regulation in morphological divergence

It is clear that the process of development is controlled by changes in gene expression. There are two types of factors that can influence the expression of a gene:

cis-acting factors – these are DNA sequences that are physically linked to the gene that they regulate. Often they are promoter or enhancer sequences that lie just upstream of a gene's coding region (that is, in the 5' UTR or flanking region). However, they may also lie within

introns, or in the 3' UTR or 3' flanking region. Typically, changes in *cis*-acting sequences only directly affect the expression of one gene.

trans-acting factors – these are DNA sequences that regulate the expression of unlinked genes. Usually, they are genes that encode transcription factors. The transcription factors are translated in the cytoplasm and return to the nucleus to bind to target *cis*-acting sequences of the genes they regulate. They may regulate many different genes on many different chromosomes. Some *trans*-acting factors may be small regulatory RNAs. Changes in *trans*-acting factors may affect the expression of many genes.

It has been proposed that morphological evolution occurs primarily through changes in *cis*-regulatory sequences. The argument behind this is that many proteins and *trans*-acting factors have multiple functions and are involved in many biological processes. Thus, changes in proteins and *trans*-acting factors are likely to have pleiotropic effects. That is, a change that is beneficial to one function or process may be detrimental to another function or process. This places strong constraints on evolution. In contrast, *cis*-regulatory changes are more independent and can lead to new expression patterns that don't interfere with other functions of the target gene.

However, there are many known examples of changes in protein sequence leading to adaptive phenotypic differences within and between species. So there remains much debate about the relative importance of *cis*-regulatory changes in evolution.

Examples:

a) wing spots in Drosophila – unlike the commonly-used laboratory fruit fly *Drosophila melanogaster*, some Drosophila species have dark spots on their wings. The spots typically occur on males and are used for courting females. The development of the spots is controlled by expression of the *yellow* gene – a dark spot forms where *yellow* is expressed. Whether or not *yellow* is expressed in the wing is determined by a *cis*-regulatory element in the 5' flanking region of *yellow*.

b) coat coloration in beach mice – different populations (or subspecies) of the mouse *Peromyscus polionotus* differ in their coat color. Mice that live in beach areas have a very light coat color that matches the beach sand; those that live further inland have a dark coat color that matches the soil. This difference is thought to be adaptive – allowing the mice to better avoid predators in their local environment. Changes in two genes are responsible for the coat color variation. One is a structural change that alters an amino acid in the melanocortin-1 receptor (Mc1r). The other is a *cis*-regulatory change that alters the expression the *Agouti* gene (which encodes a ligand for Mc1r).

References and additional reading:

N.H. Barton *et al*. (2007) *Evolution* (chap. 11). Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY.

B. Prud'homme *et al*. (2006) Repeated morphological evolution through *cis*-regulatory changes in a pleiotropic gene. Nature 440: 1050-1053.

C.C. Steiner *et al*. (2007) Adaptive variation in beach mice produced by two interacting pigmentation genes. PLoS Biology 5: e219.