



# 7 Pax and homology

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The third act in  
the history of evolution

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Genes for the development of light-sensitive cells can be found in the first single-celled organisms. From such early forms, the animal kingdom has evolved many types of eyes, all of which make use of common genetic functionality during embryonic development.

# Pax and homology

## the third act in the history of evolution

### Act one: What is evolution?

In 1856, *Charles Darwin* was the first to formulate a consistent theory of evolution by discovering the main causal factors that produce evolutionary changes in organisms, these being selection and mutation. The variety of individuals in a population provides the raw materials for a selection procedure that results in some individuals being more (and others to be less) successful at reproduction, based on momentary environmental conditions. This changes the genetic makeup of the population that follows – the quintessence of evolution.

### Act two: Modern evolutionary synthesis

The first half of the 20th century produced the so-called modern evolutionary synthesis – a combination of Darwinian theories with the laws of heredity. The evolutionary mechanisms described by Darwin – mutation, variation, heredity, and natural selection – act primarily on the level of genes and produce new adaptations and even new species over time.

### Act three: EvoDevo

An entirely different question concerns the change from an inseminated egg with a certain set of genes to a fully differentiated lifeform with a head, arms, legs, wings, and other body parts – all in their appropriate place. Modern genetics provided great advancements in this field, as did the synthesis of evolutionary theories with those of developmental biology. In fact, such deliberations have opened up an entirely new field of research

that is now called evolutionary developmental biology (often abbreviated as EvoDevo).

### All organisms are based on identically structured DNA

The number of possible genes may be very large, but not infinitely so. Some genes of microorganisms barely differ from those of humans. EvoDevo concerns the question of how such a stunning array of species could develop from a limited number of building blocks. Researchers have since discovered that the

Fruitfly (*Drosophilidae*)



successive activation of genes in the embryo is subject to strict hierarchical control.

### The time of activation is important

Due to the different times at which certain genes are activated, it is possible for a single gene to have different effects in different species. So-called master control genes are tasked with providing the impulse for the formation of entire organs.

### Hox and Pax genes regulate the fundamental body plans

The so-called Hox genes specify the length axis of the organism. At a later stage, the so-called Pax genes are of fundamental importance for the development of eyes. The Pax6 gene encodes



House mouse  
(*Mus musculus*)

Flatworm (*Plathelminthes*)

a protein that regulates the transcription factor, in other words, the activity of subordinated genes. It was soon discovered that a group of Hox genes can be found in virtually all examined multicellular (metazoan) animals, and that it may, therefore, be classified as homologous. For this reason, these genes must have been conserved over large spans of evolutionary time – at the very least since the Cambrian explosion, dating back 540 million years.

### Homeotic genes

These genes control the identity of segments and the detail of their structures. Mutations among these genes can lead to a complete or partial change of organ structures, or even of whole bodily segments. Homeotic genes were discovered due to a very conspicuous mutation in fruit flies (*Drosophila melanogaster*), which grew legs on their heads in places where their antennae should normally be. These mutants were then called “antennapedia”.

### The discovery of key genes

A homeobox is a characteristic sequence of such homeotic genes. It codes for special, delimited areas of proteins and is able to shut down whole areas of genes. From the genetic analysis of spontaneous mutations that caused fruit flies to develop without eyes, researchers were able to identify the so-called key gene based on a regulatory cascade during the development of eyes. This Pax6 gene turned out to be important for further development, as it strengthened the transcription of some genes, while restricting the transcription of others. It belongs to a whole family of regulatory genes that control a variety of developmental processes.

### Functioning eyes in the wrong place

A spectacular experiment succeeded in artificially inducing the development of additional, functioning eyes in other parts of the fruit fly body – for instance, at the antennae, at the wing basis, and on the thorax. As the genomes of many other organisms were compared – a feat that is almost routine these days – this Pax6 gene turned out to be homologous. Even animals with only very primitive eye spots (such as the primordial flatworm *dugesia spec.*, or the box jellyfish that have lens eyes at the side of their shields) were found to be under the influence of the same or of a homologous gene. This is remarkable, as the animals found today have diverged since at least the Cambrian era 540 million years ago. Despite this enormous difference in ancestry, it was possible to produce additional eyes in fruit flies using a version of the gene that originated in mice.

### The evolution of complex diversity

Many such control genes – responsible for the development of an organ and compatible with wildly divergent species – have since been discovered. These “main switches”, which govern the activity of several hundred subordinated genes, also provide an explanation for one of the most difficult problems of evolutionary biology, that being the emergence of complex diversity.

### Even single-celled ancestors have evolved a light-sensitive protein

All of these discoveries led to a new understanding of evolutionary processes in developmental biology, and may therefore, be called the *third act* in our quest to understand the history of evolution. From comparative morphology, one previously had to assume that the diversity of eyes in the animal kingdom must have evolved independently and therefore convergently. The developmental geneticist *Walter Gering* suggested a hypothesis that envisioned the existence of a common genetic basis for all types of eyes – the Pax6 gene. This would indicate a non-convergent evolutionary history of eyes. Gering claimed that light-sensitive proteins had already evolved in single-celled ancestors.

### Two types of light-sensing cells

From this ancestral material, a cell that specializes in light detection could evolve in the first multicellular animals. It carried cilium, as well as a membrane in which the photosensitive protein was embedded. The membrane was then enlarged by protrusi-

ons in an effort to increase the space available to that protein, and thus, to maximize light sensitivity. This type of receptor cell is classified as “ciliar”. Such types of cells are common in the eyes of most animals, including humans. Arthropods have developed a different type of sensory cell. They have reduced the cilium, enlarging the cell membrane instead through finger-like protrusions, known as microvilli. This produced a light sensory cell that, if aggregated into groups, formed a rhabdom. Rhabdoms function as conductors of light, and can mostly be found in the ommatidia of crabs and insects. This type of receptor cell is classified as “rhabdom-based”.

### The prototypical eye

Gehring termed an organ a “minimal eye” if it only consisted of one photo receptor cell and another cell to block incoming light. Such a photoreceptor is also called a prototypical eye. Being equipped with the pigment rhodopsin, it is already guided through its development by the Pax6 gene. Darwin himself has raised the question of how often independent eyes evolved in the various groups of the animal kingdom. He already suspected that all eyes could have originated from a prototypical version. In fact, such types of simple eyes were later discovered in flat worms. Gehring, among others, later proved this assertion, by concluding that the development of all types of eyes is controlled by the Pax6 gene – from simple pit eyes, to pinhole camera eyes, lens eyes, and the huge diversity of facet eyes.

### Pax6 is in control

Molecular studies have shown that the procedure by which eyes develop is the same in all animals – from flat worm to mammals,

including humans. About 65% of genes expressed in the retina of the fruit fly are also active in the retina of the mouse. The Pax6 master control gene stands at the top of the hierarchical cascade. Even the eye’s two-cell prototype, which can still be found in some species of flatworms, is controlled by the Pax6 gene. This system of cascading control occurs likewise in all higher lifeforms, leading to the development of very different types of eyes by the introduction of more and more genes – for instance, genes for the development of lenses.

### Opsines cause the nerve impulse

Despite this morphological diversity, similar physical and chemical principles determine the process of vision. Opsines are present as visual pigments in all multicellular organisms. These are photoreceptor proteins that when stimulated by a single light impulse (photon), experience a change in conformation, causing a nerve impulse to fire.

### Why do some large groups of animals lack eyes?

As elegant as the explanation of the embryonic development of eyes under control of Pax6 genes may seem, it does not address yet another seeming discrepancy. Indeed, many greater groups of animals lack eyes altogether, while others have developed very different types of eyes. Whether all of these types are homologous or not depends on the correct interpretation of homology, including its criteria.

### Homology – analogy

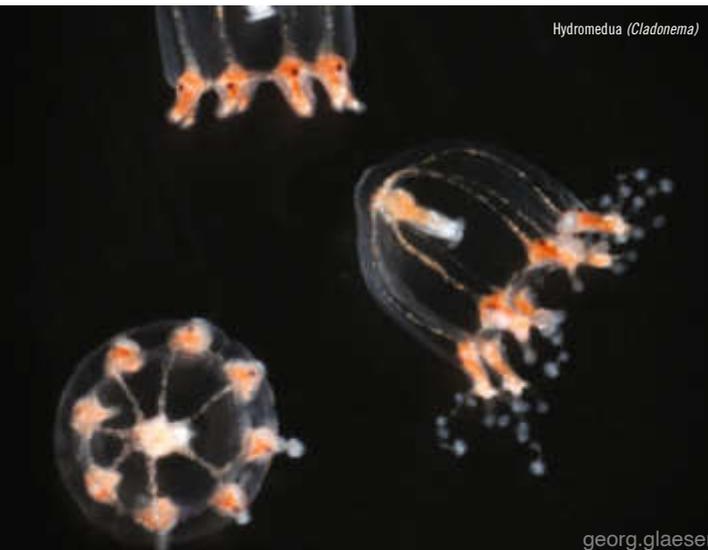
Organs, structures, physiological processes, macro molecules, and even behavioral patterns are termed homologous insofar as they exhibit such similarities in two or more species that they must have inherited said features from common ancestors. A statement about homology implies a dedicated assumption about the ancestry of the observed features.

### How to recognize homology

Three criteria must be met for homology to be properly asserted:

#### 1. The criterion of position

When comparing specific structures of different species, they should occur in analogous locations. The bones of our limbs are a very simple example of this relationship. In the front limbs of all mammals, the radius always resides between the upper arm bone (humerus) and the wristbone (carpus), regardless of



Hydromedusa (*Cladonema*)

whether the limb is dedicated to running, digging, grasping, or for the purpose of flight. Due to a shared genetic basis at play, the construction of these features during embryonic development proceeds in the same manner, and thus, these organs can be considered to be homologous insofar as they are based on the same genetic information inherited from the last common ancestor.

### 2. The criterion of specific qualities

Different organs may also be considered to be homologous if they are found to be separated from the original group. For instance, an upper arm bone is recognizable as such, even if it is not grouped with the other bones of the limb. A connoisseur is always able to identify the bone as a humerus and can often recognize it as being the humerus of a dog, a cat, or a shrew. This is possible because some organs and structures exhibit a special quality that makes them unmistakable to the experienced eye.

### 3. The criterion of continuity in alteration

The aforementioned criterion is also true for macro molecules, as it is for genes, whose specific qualities lie in the sequence of their DNA. Since organs and structures may undergo radical change over evolutionary time, a simple comparison between two species does not usually lead to a useful result. Additional species, which exhibit transitional forms, have to be considered as well. Thus, the third criterion is often called the criterion of transitional sequences. It represents the realm of comparative anatomy and physiology, which deals with the gradual changes that occur across the history of species. Usually, it involves the analysis and comparison of contemporary or fossil forms. Such methodology led to the discovery of the phylogenetic history that connects equine animals to contemporary whales.

### Not all types of eyes are homologous

The comparison of genes represents a modern method of phylogenetic research, and the conclusions that were drawn from the analysis of Pax7 genes provide a good example of this procedure. However, this does not mean that all eye types within the animal kingdom are homologous. For the development of sophisticated eyes, a cascade of further genes is required, and there is no doubt that these genes evolved much later, as well as completely independently. The Pax6 genes form the basis for the development of eyes, but the question concerning the type of

eye that ultimately emerged depends on subsequent adaptations that led to independent evolutionary lineages. This means, that a type of eye is only homologous with respect to other eyes of the same type and complexity. Facet eyes from two species are only homologous insofar as their complexity is comparable.

