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Dispatches

Opsins: Evolution in Waiting

Complete vertebrate genome sequencing has revealed a remarkable stability and uniformity in the protein-coding gene set, which at first glance might suggest that gene duplication events are relatively rare. This may be a red herring, or at least a red cichlid, as the Lake Malawi cichlid fishes show rapid and extensive duplication and diversification of their retinal cone photoreceptor opsin genes.

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"*all the organic beings which have ever lived on this earth have descended from some one primordial form, into which life was first breathed*"
Charles Darwin, 1859

Darwin reached this remarkable conclusion by observing that "*all living things have much in common, in their chemical composition, their germinal vesicles, their cellular structure, and their laws of growth and reproduction*" [1]. If Darwin had had the means to examine the heritable DNA code he would have come to the same conclusion about our genes, that homologous genes are derived from a common ancestral gene, and he would then have posed the next logical question: where did the common ancestral gene come from?

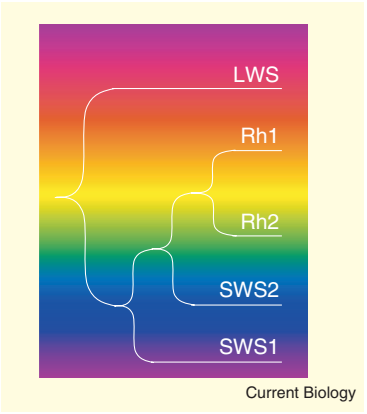

Figure 1. Phylogenetic relationships between the major vertebrate opsin gene lineages.

A series of four gene duplication events progressively produced the vertebrate LWS, SWS1, SWS2, Rh2 and Rh1 opsin genes. The position of each branch on the background spectrum approximates the spectral sensitivity of each opsin: LWS (red cone) 510–560 nm; SWS1 (UV cone) 360–430 nm; SWS2 (blue cone) 440-460 nm; Rh2 (green cone) 470–510 nm; and Rh1 (rod) ~500 nm.

From our earliest ability to decode biological information in nucleotide and amino acid sequences, it was clear that many of our genes share related sequences that group them into multi-gene families. This was evident from the globin amino acid sequences, which ultimately revealed that new genes are produced by duplication of existing genes followed by sequence and functional diversification [2]. Over time, mutation may eventually obscure gene sequence relationships, but Darwin's conclusions regarding the common ancestor of all life applies equally to the single common ancestor of all genes in the first self-replicating molecules.

The genome of the 'last universal common ancestor' (LUCA) of all life on Earth, which existed about 3.5 billion years ago, encoded the ancestral genes of many of the modern-day, ubiquitous multi-gene families. These include gene families such as the G protein coupled receptors [3]. Analysis of the sequence variation between members of a multigene family allows us to reconstruct the evolutionary history of the gene family and place time frames on gene duplication and gene loss events. This provides a window into the nature of ancestral life forms that existed hundreds or even thousands of millions of years ago and the natural selection pressures that drove evolution [4].

G protein coupled receptors detect an enormous variety of physical, chemical and biological signals and initiate signal transduction cascades to activate a cellular response [3]. Photons of light are detected by an ancient subfamily of the G protein coupled receptors: the opsin genes. In animals, photosensitivity serves many purposes, the most conspicuous of which is vision. Following the divergence of the ancestral vertebrate and invertebrate species, about 700 million years ago, the ancestral opsin gene underwent a series of independent duplication and diversification events [5]. In the early vertebrates, four successive gene duplication events produced five visual pigment opsin gene lineages [6] and the evolutionary basis of both photopic and scotopic vision (Figure 1).

Four opsin gene lineages encode the cone photoreceptor visual pigments, LWS (red), SWS1 (ultraviolet), SWS2 (blue/violet) and Rh2 (green). These opsin proteins, combined with a vitamin A-based chromophore, are responsible for bright light (photopic), colour vision. Differential sensitivity to the visible light spectrum — long wavelength (red) versus short wavelength (blue) — arises from amino acid differences in each opsin protein. The fifth opsin gene lineage, Rh1 (rod), is expressed in the rod photoreceptors and produces monochromatic, dim light (scotopic) vision [6].

The vertebrate Rh1 and Rh2 genes were produced by duplication of the ancestral Rh opsin gene approximately 500 million years ago (Figure 2), after the separation of the jawed (sharks and rays) and the jawless (lampreys and hagfishes) vertebrates [7]. So, colour vision clearly evolved first in the ancestral jawless vertebrates, with dim light vision appearing only after the evolution of the jawed vertebrates. Analysis of the genetic and functional complement of opsin genes in evolutionarily significant

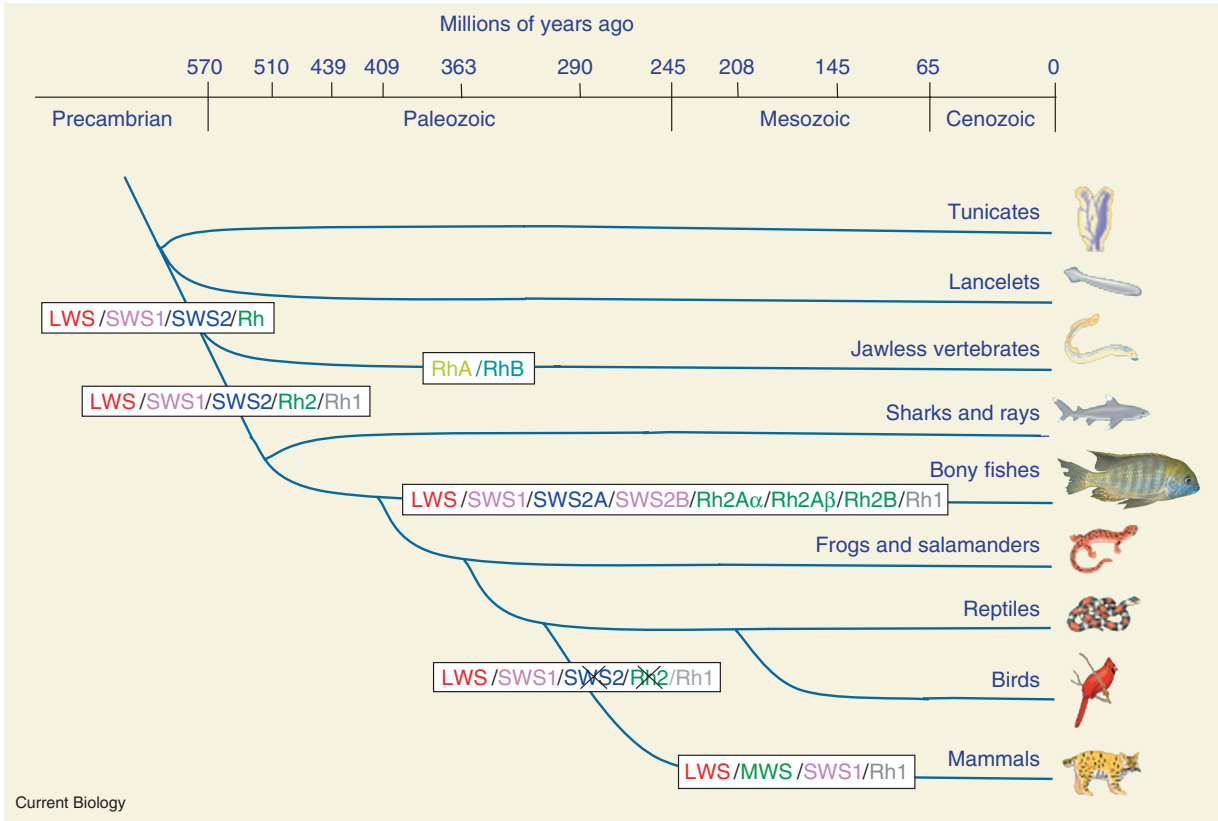

Figure 2. Reconstruction of the evolutionary history of the vertebrate opsin gene family.

Mapped on to a phylogeny of the vertebrate species are boxes showing the genomic complement of opsin genes in ancestral species to indicate time frames of gene duplication and loss events. Four opsin genes were present in the ancestral jawless vertebrate: LWS, SWS1, SWS2 and Rh. Following speciation of the jawed vertebrates, the ancestral Rh opsin duplicated to produce the Rh1 and Rh2 opsin genes in the ancestral jawed vertebrate. Independently, the Rh opsin gene duplicated to produce the distinct RhA and RhB genes in the jawless vertebrate lineage. The loss of the SWS2 and Rh2 opsin genes in the ancestral mammal is shown, as is the recent duplication of the mammalian LWS opsin to give the LWS and MWS opsins. The extraordinary rate of opsin gene duplication in the cichlid fishes is shown on the bony fishes branch. The SWS2 gene duplicated to produce SWS2A and SWS2B. The Rh2 gene underwent two consecutive duplications to firstly produce Rh2B and Rh2A, which further duplicated to generate Rh2Aα and Rh2Aβ. An approximate time line is shown. (The photograph of the cichlid was kindly provided by Karen Carleton and Ad Konings, Ad Konings/Cichlid Press.)

vertebrate species allows reconstruction of the phylogenetic history of the opsin gene family in relation to significant transitions in the evolution of the vertebrate species (Figure 2).

This relatively stable evolutionary history of the vertebrate cone and rod opsin genes is similar to many other multigene families and gives the immediate impression that gene duplication events are rare. Comparison of the completed vertebrate genome sequences identifies a high proportion of orthologous genes. An alternative interpretation would be that gene duplication events occur at a high rate but the overwhelming majority are not transmitted due to selection pressure [8].

A study reported in this issue of *Current Biology* [9] provides evidence in favour of the latter interpretation. Jim Bowmaker and colleagues [9] have characterised the functional and genetic complement of cone photoreceptor opsin genes in four species of Lake Malawi cichlid fishes. All species harbour at least seven cone opsin genes that were generated by a series of consecutive gene duplication events within both the Rh2 (green cone) opsin gene lineage and the SWS2 (blue cone) gene lineage. Microspectrophotometry of isolated cone photoreceptors and *in vitro* reconstitution of cloned opsin genes confirmed that all seven cone opsin genes are functionally expressed. But different Lake Malawi cichlid species differentially and preferentially express only three of the possible seven cone opsin genes at any one time [9]. So, what are the factors that may have given rise to this rapid expansion in the hard-wired genetic diversity of cichlid cone opsin genes?

The cichlid populations of the African Great Lakes (Lake Victoria, Lake Malawi and Lake Tanganyika) have undergone the most rapid and species-dense adaptive radiations that we know about. Ancestral colonising populations migrated out from Lake Tanganyika into the other lakes and, in the last 100,000 years, have expanded and separated into about 500 new cichlid species in Lake Victoria. Over the last ~250,000 years the colonising population in Lake Malawi has produced approximately 1000 cichlid species [10].

The rapid rates of speciation in these cichlid fish populations are

equally matched by their spectacular diversity in morphology and body colouration [10]. As well as expanding into unoccupied ecological niches, sexual selection of body colour variation among males, by female cichlids, is thought to contribute to the incredibly fast rates of speciation [10]. Sexual selection may be an even more important factor in driving rapid speciation in the clear-water environment of Lake Malawi. It would be very interesting to compare the spectral composition of body colour patterns with the maximal spectral sensitivities of the expressed cone opsins in the Lake Malawi cichlids. Selection pressure to match, or tune, the visual sensitivities of the opsins to the local light environment may also contribute to speciation.

In the area of visual ecology, there are many well-established correlations between spectral composition of the light environment and maximal spectral sensitivities of visual pigments [11]. For example, deep-sea fish live in a short wavelength-rich light environment, which is matched by a short wavelength shifted maximal sensitivity of their Rh1 opsin and rod photoreceptor [11].

Careful analysis of the opsin genes of the Lake Malawi (clear water, short wavelength-rich) and Lake Victoria (turbid water, long wavelength-rich) cichlids has revealed positive selection in opsin genes in species living in the two different environments [12]. This is important, as it takes the numerous observations of matched light environment and visual sensitivities beyond a series of interesting correlations and demonstrates positive selection and adaptive, photic environment-driven evolution in the East African cichlids.

From genome sequencing data, the rate of gene duplication has been estimated at 0.01 duplications per gene per million years [8]. This is much higher than the observed number of duplicated genes because gene gain is generally balanced by a short half-life of new genes and a high rate of gene loss [8]. The identification of seven cone opsin genes [9] and therefore the potential for heptachromatic vision in the Lake Malawi cichlids opens up a Pandora's box of untapped evolutionary potential that is constantly creating new genes.

The present evolutionary circumstances of the cichlid fish populations in the East African Lakes is providing a window into how rapidly molecular evolutionary mechanisms can kick into top gear, switch the balance towards keeping those newly duplicated genes and exploring new areas of genetic space. This type of 'evolution in waiting' may ultimately govern the expansion and diversification of whole populations to exploit newly available ecological niches through rapid speciation.

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Axon Pruning: *C. elegans* Makes the Cut

Axon pruning has recently been described in the simple nervous system of the nematode *Caenorhabditis elegans*. Generating excess processes and pruning may be a phylogenetically conserved feature reflecting a flexibility to modify neural circuits.

William G. Wadsworth

Axon pruning is a means to modify the patterning of axons in a nervous system. For example, during embryonic development a transient nervous system arises that may contain excessive and unnecessary projections. Axon pruning is one means to eliminate inappropriately made connections and to help transform the nervous system into a mature state containing specific patterns of neural circuits [1].

This pruning of axons has been observed in different organisms when the nervous system becomes remodeled. During the development of layer V of the mammalian cortex, for example, different classes of neurons initially send axon branches to both the spinal cord and the superior colliculus. Later, the motor cortical neurons selectively prune their branches to the superior colliculus, while visual cortical neurons selectively prune their branches to the spinal cord [2] (Figure 1A). Axon pruning is also observed in holometabolous insects such as *Drosophila* and *Manduca sexta.* In such insects the nervous system is extensively reorganized during