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Primer

Evolution of vertebrate visual pigments

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Vision endows an animal with the ability to detect almost instantaneously the environment around it. A primary visual function must be the detection of objects against a background, but a visual system based solely on luminance differences can be confused if the brightness of either the object or the background is highly variable. This occurs, for instance, in shallow waters where surface wave ripples and reflections from the substrate can cause continuously variable luminance.

Such potential confusion can be overcome by adding a further dimension to the visual system, the ability to detect differences in the spectral composition of the environment, where spectral reflectance (colours) will be independent of luminance: this is therefore a possible explanation for the evolution of colour vision so early in vertebrate evolution.

The vertebrate retina contains two types of photoreceptor: rods that function at low light levels and mediate scotopic vision, and cones that function in daylight, mediate photopic vision and provide the basis for colour vision. Most vertebrates possess only a single class of rod and scotopic vision is monochromatic. In contrast, colour vision requires at least two spectrally distinct classes of cone combined with a nervous system that can compare the quantum catch of one class of cone with the quantum catch of another.

Rods and cones contain visual pigments that are composed of a protein moiety, opsin, linked to retinal, a derivative of vitamin A. Opsins are members of an extensive family of G-protein-linked membrane receptors that are composed of about 350 amino acids which form a palisade of seven α-helical transmembrane regions enclosing a ligand-binding pocket (Figure 1). Retinal is bound into the pocket through a Schiff base linkage to a lysine residue in the seventh helix. The spectral sensitivity of the pigment is then determined primarily by the interactions of retinal with specific amino acids lining the ligand-binding pocket (Figure 1).

Evolutionary origin of visual pigments

Comparative studies across all of the major vertebrate groups have established that, in addition to a rod class of pigment, there are four spectrally distinct classes of cone pigment encoded by distinct opsin genes: a long-to-middle wave class (LWS) maximally sensitive in the red-green spectral region from about 490–570 nm; a middle-wave class (RH2) sensitive in the green from about 480–535 nm; a short-wave class (SWS2) sensitive in the blue-violet from about 410–490 nm; and a second short-wave class (SWS1) sensitive in the violet-ultraviolet from about 355–440 nm.

These cone classes have arisen through a series of gene duplications from a single ancestral opsin gene. By applying estimates of the rate of gene divergence, it is suggested that the appearance of the four classes occurred very early in vertebrate evolution, about 450 million years ago. This is close to the time of one of the major steps in vertebrate evolution, the appearance of jaws. Primitive jawless fish, agnaths, are represented today by lampreys and hag fish, and recent studies have established that, at least in lampreys from the Southern hemisphere, functional genes from all four cone opsin classes are present.

From this, it is clear that the cone opsin genes evolved before the evolution of jaws, implying that primitive jawless fish of the shallow Ordovician seas possessed four spectrally distinct cone classes and thus had the potential for a tetrachromatic colour vision system.

Spectral tuning

Modern vertebrate groups, most notably diurnal reptiles, birds and shallow water teleosts, express at least one gene from each of the four cone opsin classes. Animals have evolved their visual sensitivity to match aspects of their photic environment, and it is likely that the primary adaptive selective pressure is the spectral range and intensity of daylight.

At the most basic level, nocturnal animals have rod-dominated retinas, whereas diurnal species have cone-rich retinas. However, visual sensitivity can be adapted, at the receptor level, to specific spectral regions and/or specific visual tasks by spectrally tuning the sensitivity of the visual pigments and/or by varying the number of spectral classes of cone.

These two tuning mechanisms are not mutually exclusive. First, mutations within an opsin gene can lead to a spectral shift in the sensitivity of the pigment. A single nucleotide substitution may lead to the replacement of an amino acid that alters the interaction between the chromophore and opsin, leading to a spectral shift.

The change in spectral sensitivity arising from a single amino acid substitution may be only a few nanometres, but can be greater than 60 nm. Additional mutations can lead to further spectral shifts, but there are only a limited number of sites within opsin that can be altered without producing a non-functional pigment.

Secondly, a species may modify the ancestral vertebrate pattern of four spectrally distinct cone classes either by the loss of one or more of the cone classes or by gene duplication, where multiple copies of one or more cone classes may be present. Mutations in the duplicated genes can then lead to the generation of two or more spectrally distinct pigments within a single opsin class.

Adaptations to aquatic environments

The aquatic environment offers a singular prospect for studying the evolution of colour vision in

vertebrates because of the great variations in underwater light. Water colour in the seas can vary from clear blue oceanic water to green coastal waters, and freshwaters show an even wider range of colours due to dissolved organic material, ranging from clear blue lake waters through to heavily peat-stained brown river waters.

As a consequence of this, the cone complement of teleost fish retinas is highly variable, ranging from species that possess cones from a single spectral class to others possessing representatives from all four classes. In addition, different morphological types of cone are present, with at least two types of single cone and double cones in which the two halves may be spectrally identical or spectrally different.

A striking example of cone pigment variation is the cichlid fish population of the East African Rift Lakes, which are renowned for their diversity, particularly in their colour patterns and sexual dimorphism. Lake Malawi has more than 700 species of cichlids that have evolved from a common ancestor within the last million years.

Typically, these cichlids have three cone visual pigments, with non-identical double cones maximally sensitive at longer wavelengths, and shorter wavelength-sensitive single cones. Fish inhabiting rocky and sandy habitats may have significantly different cone visual sensitivities. Some rock-dwelling species have double cones with maximum sensitivities at about 535 and 490 nm and ultraviolet- or blue-sensitive single cones with λ_{max} at about 370 or 420 nm (Figure 2D), whereas other sand-dwelling species are less sensitive to short wavelengths, possessing double cones with maximum sensitivities at about 570 and 535 nm and blue-sensitive single cones with λ_{max} at about 450 nm (Figure 2C). In total, across species, seven spectrally distinct cone classes have been identified (Figure 2).

The genes for these seven pigments represent the four major classes of cone opsin, with gene duplications in the SWS2 and RH2 classes (Figure 2B). The remarkable feature of vision in African cichlids, however, is that spectral sensitivity is determined by the differential expression of primarily only three of these seven available cone opsin genes.

This raises the important question as to how all seven of these genes are maintained. Certainly in some species, although only three genes are primarily expressed, the remaining four are expressed at low levels, though the significance of this to visual function is not clear. Further, in some species it is likely that there are switches in gene expression during development, with larval stages expressing shorter wave and adult fish expressing more longer-wave pigments. Phylogenetic analysis of opsin genes from a wide range of

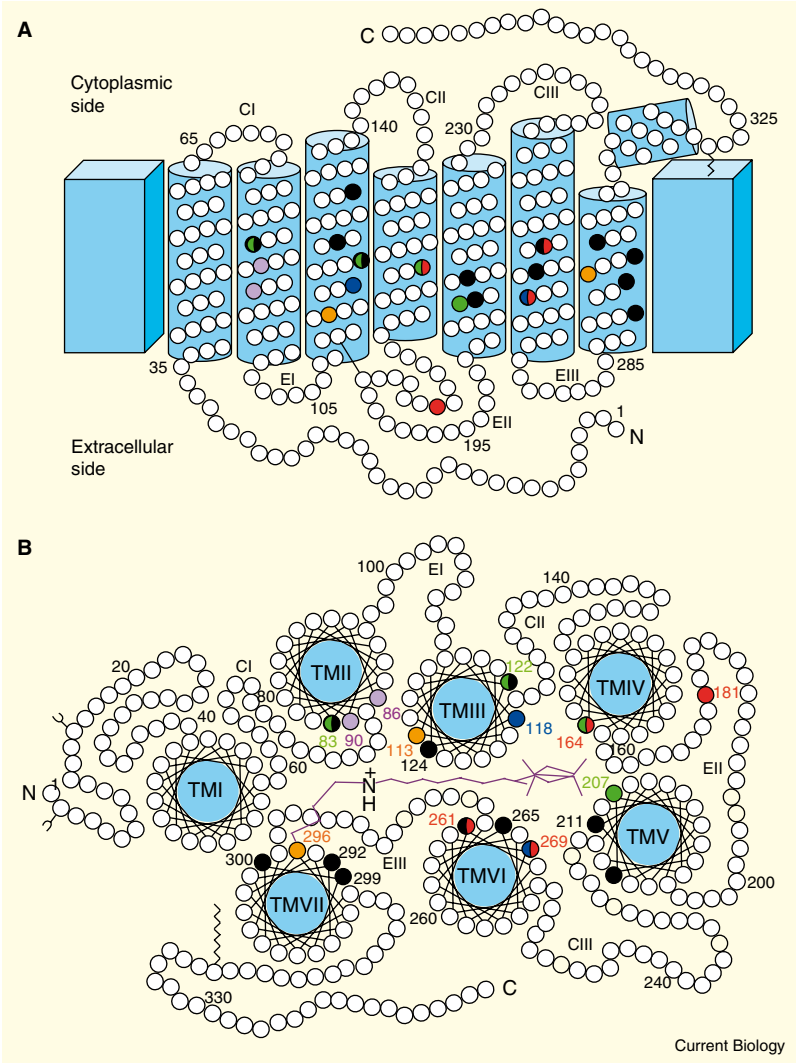

Figure 1. Schematic diagrams of a visual pigment molecule.

(A) Two-dimensional diagram illustrating the seven transmembrane α-helices. (B) View showing the arrangement of the helices around the chromophore, retinal, shown in purple (basic design kindly supplied by W.L. Davies). Although the helices are of different lengths, for simplicity, each helix is shown with only the central 18 amino acids. The numbering is based on mammalian rod opsin. Lysine 296 (orange) is the binding site of retinal and glutamate 113 (orange) provides the Schiff base counter ion. Major sites involved in spectral tuning are colour coded with opsin class: LWS red, RH2 green, SWS2 blue, SWS1 violet and RH1 black. Split colours indicate sites involved in tuning in more than one opsin class. Note how sites tend to cluster around either the Schiff base linkage or the ionone ring of retinal.

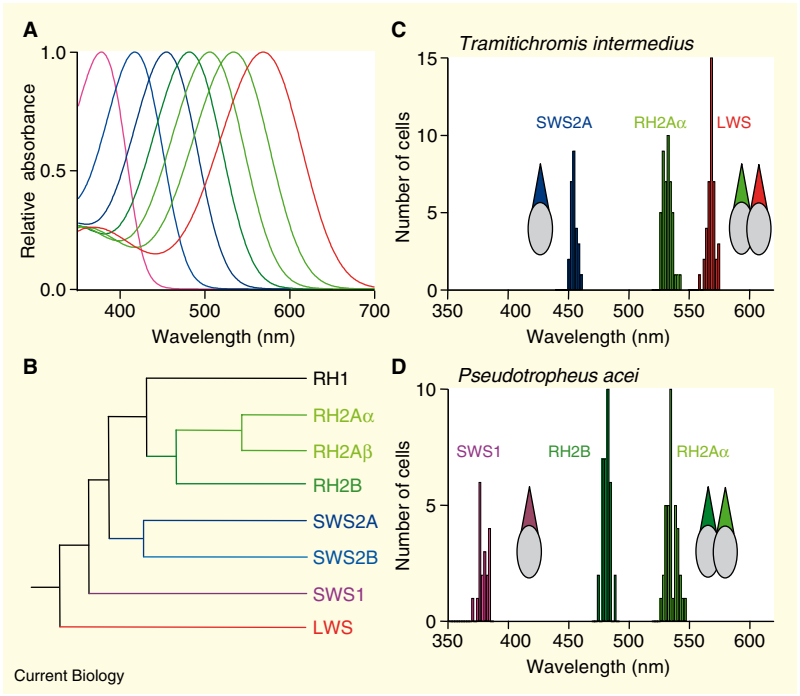

Figure 2. The organisation of the seven cone opsin genes present in the species flocks of African cichlid fish.

(A) Absorbance of the seven cone pigments colour coded with opsin class. (B) Schematic of the phylogenetic arrangement of the opsins illustrating the gene duplications that have occurred within the SWS2 and RH2 opsin classes. The recent duplication within the RH2A class may be restricted to African cichlids. (C,D) The distribution of the dominant spectral cone classes in two different species of Malawi cichlids as measured by microspectrophotometry. The two species illustrate differential expression of three of the seven cone opsins present in the cichlids.

different teleost groups suggests that this opsin arrangement is not restricted to African cichlids, but may be a feature of all percomorph fish.

Classic examples of spectral tuning of visual pigments within specific opsin classes which correlate with photic environments are provided by deep water teleost fish. Water acts as a monochromator, so that with increasing depth there is a gradual reduction, not only in the intensity of the light, but also in the spectral range. This is most significant at longer wavelengths, which are rapidly absorbed. Light penetrates clear water to a maximal depth of about 1000 m where it is restricted to a narrow band centred in the blue around 480 nm.

The visual systems of deep-sea fish show numerous adaptations to this photon-limited environment. These include the loss of cone photoreceptors and the shortwave shift of the λ_{max} of the rod pigment from around 500 nm to 480 nm or less. Sensitivity to this shorter wavelength matches the wavelength of maximal spectral transmission of oceanic waters and the blue bioluminescence emitted by photophores present on many species of deep-sea fish.

Surveys of large numbers of deep-sea species suggest that the peak sensitivities of rod pigments cluster at specific spectral locations between about 450 and 500 nm, the clusters being separated by between 5 and 10 nm, with the vast majority of pigments centred at 477 and 483 nm. Across a number of diverse species, these shortwave shifts arise from amino acid substitutions at just eight sites within the opsin protein (Figure 1).

A similar situation exists in the unique environment of Lake Baikal in central Siberia. Lake Baikal is the deepest lake in the world at over 1600 m and is unique in that its waters are fully oxygenated even in the deepest regions. This has allowed both invertebrate and teleost species flocks to evolve throughout the depth of the lake.

Some 25 species of cottoid fish are endemic to the lake and they can be divided into groups that occupy progressively deeper habitats. Littoral species living in the surface waters have relatively cone rich retinas with three spectral classes of cone (Figure 3). Species occupying deeper waters, however, have lost the long-wave-sensitive cones and, with increasing depth, they show a series of short-wave shifts in λ_{max} in the middle- and short-wave cones as well as in the rods. The middle wave cones (RH2A) fall into four clusters with λ_{max} ranging from about 525 nm to 500 nm, and the short-wave cones (SWS2A) shift from about 450 nm down to about 430 nm (Figure 3).

The rods similarly show distinct spectral steps, with λ_{max} shifting from about 516 nm in the littoral species down to 484 nm in abyssal species. Comparisons of the derived amino acid sequences of the rod pigments have identified just four amino acid substitutions that can account for these shifts. These same substitutions are also found in deep-sea fish rod pigments. The effect of each substitution on λ_{max} is approximately additive and each corresponds to a particular lineage of evolution (Figure 3). Similarly, the spectral shift in the SWS2A pigments from 450 to 430 nm is largely due to a single amino acid substitution.

Sensitivity to ultraviolet

Sensitivity to short wavelength light is conferred by the SWS1 class of cone pigments. The ancestral form of this pigment was almost certainly ultraviolet-sensitive and UV-sensitivity remains relatively common in vertebrates. In many species, however, the λ_{max} of the SWS1 pigment has long-wave-shifted to the violet region of the spectrum with peaks between 390 and 440 nm, depending on species. In

many cases, a single amino acid replacement is responsible for this change (either at site 86 or 90, see Figure 1), although in primates, additional changes appear to be required.

The evolution of violet-sensitive pigments from UV-sensitive pigments has occurred separately on many occasions in vertebrate evolution, although it has yet to be reported in teleost fish where violet-sensitive pigments are short-wave shifted duplicate copies of the SWS2 cone class.

Cone classes in mammals

One of the most striking modifications of the ancestral pattern of four spectral classes of cone opsins is found in mammals, where only the two spectrally extreme classes are present and the RH2 and SWS2 have been lost. In general therefore, but with notable exceptions as discussed below, mammals are dichromats and may be considered colour deficient in comparison to many diurnal lower vertebrates.

The most likely explanation for this loss is related to the evolutionary history of mammals. Primitive mammals evolved from reptilian ancestors in the Cretaceous about 100–150 million years ago and went through a long nocturnal phase. Presumably, as nocturnal animals, they lost the sophisticated tetrachromatic colour vision system of their ancestors, retaining only the minimum requirement for colour vision: two cone classes supporting dichromatic colour vision. By retaining the LWS and SWS1 cone classes, they also preserve a broad photopic spectral sensitivity.

Within these two remaining cone classes, there is a wide spread of cone spectral maxima. The SWS1 pigments have λ_{max} ranging from the ultraviolet around 365 nm, in a number of small rodents such as mice and hamsters, to about 440–450 nm in many ruminants, carnivores and rodents such as squirrels. Similarly, the LWS pigments have λ_{max} ranging from as short as about 510 nm in mouse to around 555–565 nm in carnivores and primates.

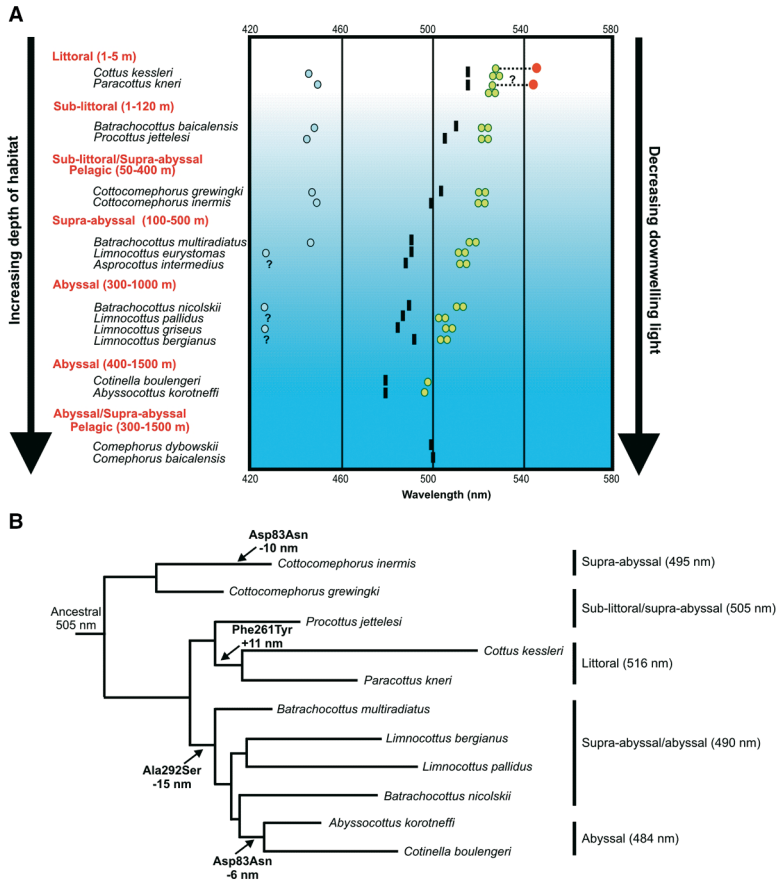

Figure 3. The distribution of visual pigments in the cottoid species flock of Lake Baikal.

(A) Spectral locations of the LWS, RH2, SWS2 and RH1 pigments from a range of species living at different depths in the lake. Red/green circles, LWS/RH2 double cones; twin green circles, RH2 double cones; blue circles, SWS2 single cones; black symbols, rods. Note the spectral shift to shorter wavelengths in all pigment classes with increasing depth of habitat. The LWS opsins are present only in surface living species. (B) Phylogenetic arrangement of species showing the specific amino acids involved in spectral tuning the rod (RH1) pigments to different wavelengths. Note, only three amino acid sites are involved, with the exchange at site 83 occurring independently in two separate evolutionary lines.

The dramatic shift from longer wavelengths to around 510 nm, as found for example in the mouse, is achieved, not by amino acid substitutions in the helices surrounding the retinal binding pocket, but by a substitution in the extracellular loop connecting helices 4 and 5 of the opsin which eliminates a chloride binding site (site 181, Figure 1).

Trichromacy in marsupials

Somewhat surprisingly, recent studies of the spectral characteristics of photoreceptors in four Australian marsupial species representative of the two major marsupial taxonomic divisions — the polyprotodont fat-tailed dunnart and bandicoot, and the diprotodont honey possum and quokka — have indicated that trichromacy may be present in these species.

Three classes of cone photoreceptors have been identified in marsupials by microspectrophotometry, maximally sensitive in the UV, green and red. Molecular analysis has shown that SWS1 and LWS pigments are present in the former and latter cone classes, but the pigment class at middle wavelengths has yet to be identified. The intriguing possibility is, therefore, that in marked contrast to placental mammals, the RH2 opsin gene has been retained and is expressed in these marsupials.

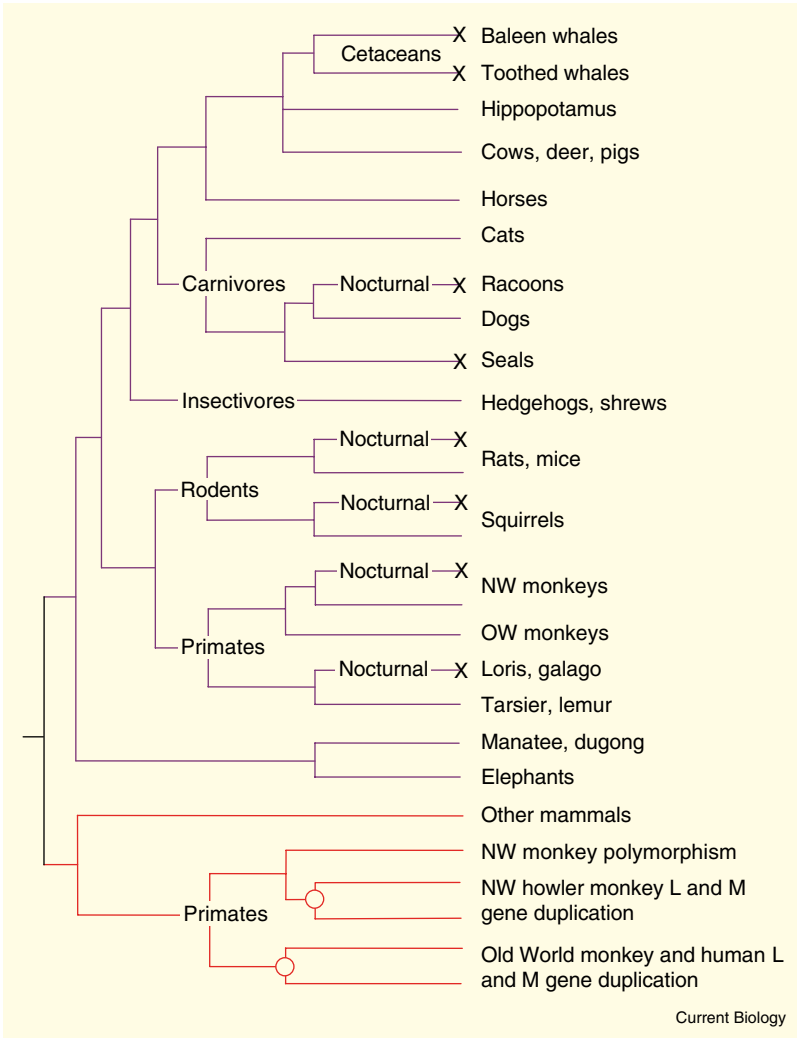

Figure 4. A highly schematic representation of the distribution of LWS and SWS1 cone opsins in mammals.

The phylogenetic tree shows only groups where information of SWS1 loss is available. The LWS opsin is probably expressed in all mammals and gene duplication in Old World monkeys and New World howler monkeys has led to trichromacy in these species. The SWS1 opsin fails to express as a cone pigment probably in all marine mammals (whales and seals), but is expressed in the aquatic manatees and dugongs. Most of the other mammalian groups in which SWS1 cones are absent tend to be nocturnal.

As yet however, and despite substantial efforts, an RH2 gene has not been identified.

Monochromacy in mammals

There are two notable divergences away from the general dichromatic mammalian pattern: the loss of SWS1 cone pigments in marine mammals and some nocturnal terrestrial species, and the re-evolution of trichromacy in primates (Figure 4).

Mammals completely lacking SWS1 cones possess only the LWS cone pigment and are thus cone monochromats, precluding cone-based colour vision. These species include representatives from most major mammalian groups (Figure 4). In these species, the SWS1 opsin gene is present, but suffers from amino acid substitutions and/or deletions that render the expressed protein non-functional. As these genetic alterations have occurred in such a wide range of species, they must have occurred independently several times during mammalian evolution.

Why these losses have occurred is not immediately apparent. Superficially, as these animals are all nocturnal, it could be concluded that colour vision is of little functional significance and that the loss of the SWS1 cones is therefore of little consequence. However, many of these species have close relatives that are also nocturnal but retain both cone types and presumably exhibit dichromacy.

Whereas S-cone loss is relatively rare among terrestrial species, it appears to be universal in marine whales and seals. These species have retinas dominated by rods and contain only a very small percentage of LWS cones. Molecular analyses in several species of whale, both baleen and toothed, have identified one or more mutations that indicate that their SWS1 opsin genes are unable to code for functional visual pigment proteins.

The phylogenetic distribution of some of these mutations indicates that they probably occurred before the divergence of the two groups of whales. Since the closest terrestrial relatives of the seals and whales (carnivores and hippopotamus, respectively) possess both LWS and SWS1 opsin genes, the mutations in the SWS1 gene in these two distinct orders of marine mammals illustrates convergent evolution, suggesting a common selective pressure. However, it is not clear what that selective pressure was. Most fish have retained short-wave cones and, as described above, water transmits primarily blue/green light, so the loss of SWS1 cones is somewhat counter intuitive.

Trichromacy in primates

Unlike most other mammals, primates are trichromatic with three cone pigments, an SWS1 pigment and two variants of the LWS pigments. In Old World primates, two different forms of the LWS gene are present, which have arisen by duplication of an ancestral gene (Figure 4). These variants show close identity with each other and encode L and M pigments with λ_{max} values of around 563 nm and 532 nm, respectively.

As all Old World primates show this duplication, it must have occurred at the base of

the catarrhine lineage around 30 million years ago. The duplicate genes form an array on the X chromosome, with additional duplicate copies of the M gene common in humans. The array is bounded on the upstream side by a so-called locus control region (LCR), the presence of which is critical for the expression of either gene. The spectral difference between the L and M pigments is largely determined by amino acid changes at only three sites (164, 261 and 269, Figure 1).

Red/green colour vision is much more variable in New World primates. Most New World species exhibit a trichromacy that is based on only two opsin genes, an autosomal SWS1 gene as in Old World primates, and a polymorphic X-linked LWS gene with multiple allelic forms that encode pigments with differing λ_{max} values lying between about 535 and 565 nm. Platyrrhines thus lack the routine trichromacy of Old World primates, as male monkeys can combine the SWS1 gene with just one of the different allelic forms of the LWS X-linked gene and are therefore dichromats. In contrast, those females that inherit a different form of the LWS gene from each parent have the bonus of trichromatic vision, because X-inactivation will ensure that only one allele is expressed per cell.

A major exception to this polymorphism-based trichromacy in New World primates is found in the howler monkey. In this species, separate L and M genes are present (Figure 4), and expressed in separate cone populations with trichromacy present in both males and females. The duplication of the LWS gene differs from that in Old World primates and appears to be limited to the howler monkey, as it is not present in two closely related species, the spider monkey and the woolly monkey, which both possess a polymorphic LWS gene.

Trichromatic colour vision in monkeys probably evolved from an ancestral dichromacy present within the arboreal environment of early primates, where the driving force was the ability to distinguish the redness of ripe fruits or reddish young leaves from a green background of foliage of highly variable luminance.

Nevertheless, the complement of just three cone pigments in Old World monkeys may be considered somewhat limited in comparison to the complexity of cone pigments available to many lower vertebrates. The basic tetrachromatic system that evolved very early in vertebrate evolution has been adapted to a great range of photic environments, perhaps reaching its most advanced forms in diurnal birds and shallow water teleosts. In these species, spectral sensitivities range from the ultraviolet to the far red and in the case of some teleost fish, gene duplications have provided a wide palette of spectrally distinct pigments from which to differentially tune their colour vision.

Further reading

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Gorilla susceptibility to Ebola virus: The cost of sociality

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Since 1994, there have been nine human Ebola-Zaire virus (EBOV) outbreaks in eastern Gabon and northwestern Congo [1–3]. A majority of them originated from the handling of ape carcasses found by local hunters [4]. The impact of Ebola-Zaire virus on great ape density is suspected to be high [2,5,6], but neither the demographic consequences of outbreaks nor the way the virus spreads within an ape population are well known. The large population of western lowland gorillas, *Gorilla gorilla gorilla*, monitored since 2001 at the Lokoué clearing, Odzala-Kokoua National Park, Congo, was affected in 2004, providing us with the opportunity to address both questions using an original statistical approach mixing capture–recapture and epidemiological models. The social structure of gorillas strongly influenced the spread of EBOV. Individuals living in groups appeared to be more susceptible than solitary males, with respective death rates of 97% and 77%. The outbreak lasted for around a year, during which gorilla social units (group or solitaries) got infected either directly from a reservoir or from contaminated individuals.

The swampy clearing of the Lokoué site (0°54.38N, 15°10.55E) is exceptionally attractive for gorillas. During a 17 month study in 2001–2, 377 gorillas, of which 92% lived in groups and 8% were solitary males, were individually identified [7]. The first evidence for the presence of Ebola among Odzala apes was the discovery of an EBOV-positive gorilla carcass