Evolution of colour vision in vertebrates

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Abstract

The expression of five major families of visual pigments occurred early in vertebrae evolution, probably about 350–400 million years ago, before the separation of the major vertebrate classes. Phylogenetic analysis of opsin gene sequences suggests that the ancestral pigments were cone pigments, with rod pigments evolving last. Modern teleosts, reptiles and birds have genera that possess rods and four spectral classes of cone each representing one of the five visual pigment families. The complement of four spectrally distinct cone classes endows these species with the potential for tetrachromatic colour vision. In contrast, probably because of their nocturnal ancestry, mammals have rod-dominated retinas with colour vision reduced to a basic dichromatic system subserved by only two spectral classes of cone. It is only within primates, about 35 millions years ago, that mammals 're-evolved' a higher level of colour vision: trichromacy. This was achieved by a gene duplication within the longer-wave cone class to produce two spectrally distinct members of the same visual pigment family which, in conjunction with a short-wavelength pigment, provide the three spectral classes of cone necessary to subserve trichromacy.

Key words Colour vision, Cone, Evolution, Retina, Rhodopsin

The evolution of vertebrate colour vision, or more fundamentally, the evolution of visual pigments and photoreceptors, has long held the interest of zoologists and visual scientists. With the great expansion of molecular genetics and the ability to identify and sequence opsin genes, there has been a reawakening of this interest.

One of the great comparative visual scientists, Gordon Walls, wrote in 1942, in his tome *The Vertebrate Eye and its Adaptive Radiation:*

'It seems necessary to believe that human colour vision owes nothing whatever to the product of the teleost and the reptile. But "human" colour vision is already present far below man in the anthropoid stock. It is not necessary to suppose that human colour vision has evolved wholly within the genus *Homo*.'

It is now possible, more than 50 years later, to suggest that in one respect Walls was wrong, but in another, partly correct. Our underlying mammalian dichromacy owes *everything* to our teleost (more strictly, fish) and reptilian ancestors (as ancient as 300–400 million years ago, MYA), but our recent acquisition of trichromacy (about 35 MYA) is a feature not only of anthropoids but of Old World monkeys in general and even some New World monkeys. The evidence to support these assumptions is derived from three comparative sources: (i) present-day 'representatives' of ancient ancestral vertebrates, (ii) modern representatives of the major vertebrate classes: teleosts, reptiles, birds and mammals, and (iii) the molecular genetics of visual pigment protein genes.

In this brief review it is not possible to give a complete survey of all the relevant published data, but only to select certain aspects that underlie and demonstrate the basic assumptions surrounding the evolution of visual pigments and colour vision. The consequence of such an eclectic selection is that much detail must be passed over and that generalisations will inevitably introduce inaccuracies.

Early vertebrates

Three questions are often asked with respect to the evolution of the vertebrate retinal photoreceptor system. First, which are the ancestral (more primitive?) photoreceptors: rods or cones? Secondly, what was the ancestral visual pigment? And thirdly, when did colour vision evolve? There are no simple answers to these questions, though an answer to the first might suggest an answer to the other two.

Rods are more conserved throughout the vertebrates, in terms of both structure and visual pigments, whereas cones are highly diverse in structure and can be divided into many spectrally distinct classes (possibly as many as five) within a given species. Rods could then be considered the simpler, and therefore ancestral, photoreceptor. However, in other respects rods could be considered to be more specialised than cones: they have a more complex morphology with isolated discs and are more sensitive, being capable of signalling the detection of a single photon. A possible way

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to approach the question of the ancestral vertebrate photoreceptor is to look at modern species that are thought to be direct descendants of the earliest of vertebrates.

The present-day cyclostomes, i.e. lampreys and hagfish, are considered to have arisen directly from ancient ostracoderms, extinct jawless, fish-like primitive vertebrates of the Silurian and Devonian periods. There has been much debate as to whether the photoreceptors of lampreys are rods or cones (see Crescitelli[1]), though it is clear that they have two classes of photoreceptor, one with long outer segments and the other with short outer segments. Both types of outer segment are morphologically cone-like in having many discs that are infoldings of the outer plasma membrane, but recent studies[2-4] clearly demonstrate that the two classes are functionally different.

Microspectrophotometry[2] has revealed that the shorter outer segments have a wavelength of maximum absorbance (λ_{max}) close to 517 nm, whereas the longer contain a pigment with λ_{max} at about 555 nm. Further, the spectral sensitivity of the dark-adapted eye is maximal at about 510–520 nm, but with short-wave background illumination the maximum sensitivity is displaced to about 555 nm. These data strongly imply that the lamprey has a duplex retina with both cone-like and rod-like photoreceptors and exhibits a Purkinje shift between scotopic and photopic vision similar to that seen in most vertebrates. However, the rod-like photoreceptors also have cone-like features: the pigment reacts with hydroxylamine in a similar manner to cone pigments,[5] the cells apparently do not saturate at high light intensities and are also involved in photopic vision, perhaps even subserving a dichromatic colour vision system.[2] Immunocytochemical reactions also support the idea that the shorter of the two outer segment types is rod-like and the longer cone-like.[3,4]

Rod opsin-like genes have been isolated and sequenced for the river lamprey, Lampetra japonica,[6] and from the marine lamprey, Petromyzon marinus.[7] The deduced amino acid sequences show about 92% similarity and have about 80% identity with rod opsins from higher vertebrates, but only about a 45% identity with cone opsins. These rod opsins are presumably expressed within the 'short', more rod-like photoreceptor. The gene sequence for the longer-wave visual pigment has not so far been published, but in P. marinus the visual pigment is sensitive to the concentration of Cl⁻ ions.[8] Under normal saline conditions the pigment has λ_{max} close to 600 nm (a porphyropsin, visual pigments based on vitamin A_2), but the removal of Cl⁻ ions displaces the λ_{max} to about 550 nm. This ionochromic displacement to shorter wavelengths appears to be characteristic of the long-wave visual pigments (with λ_{max} longer than about 520 nm) of all vertebrate groups[9-13] and is controlled by a chloride binding site composed of two positively charged amino acids, lysine and histidine, on the second extracellular loop of opsin. The chloride binding site is absent from some mammalian long-wave cone pigments that have λ_{max} closer to 500 nm.[14]

A conclusion that can be drawn from these studies of lamprey photoreceptors is that the ancestral vertebrate visual system was based on relatively unspecialised photoreceptors (perhaps more cone-like) and that at least two spectral classes of photoreceptor were present at a very early stage. The presence of the two classes of visual pigment suggests a gene duplication early in vertebrate evolution (about 400 MYA) to give a middle-wave-sensitive (MWS), but non-chloride-sensitive visual pigment and a longer-wave-sensitive (LWS), chloride-sensitive pigment, the chloride binding being necessary to spectrally tune a visual pigment to longer wavelengths. There is no reason why an ancestral vertebrate should not have been carrying more than a single opsin gene and colour vision (requiring two spectral classes of photoreceptor), as distinct from photopic vision, may have been present in the earliest verterates.

A second group of modern representatives of vertebrate ancestral forms is the sturgeons (Chondrostei), which are considered to be degenerate ray-finned fish arising from ancestral Devonian forms (about 350 MYA). These fish have a clearly duplex retina with rods and at least three spectrally distinct classes of cone (with oil droplets). The cones (containing porphyropsins in Acipenser transmontanus) have λ_{max} at about 610, 540 and 465 nm, whereas the rods have λ_{max} at about 540 nm. Sturgeons have colour-opponent horizontal cells (with possible up to six classes in Acipenser baieri), somewhat similar to teleosts.[15-18] These data suggest that at a very early stage in vertebrate evolution there were at least four opsin families (a rod and three cones) with the neural mechanisms to give at least trichromatic colour vision.

Teleosts

Teleost fish (having evolved in the last 150 MY) show a great diversity of visual pigments and colour vision depending on their environment and life styles (for a review, see Bowmaker[19]), but in diurnal species living in relatively shallow water where a broad spectrum of light is available there are, in addition to rods, at least four spectral classes of cone including a violet- or ultraviolet-sensitive (V/UVS) class. This arrangement appears common amongst cyprinids, such as the goldfish, which has cone pigments (porphyropsins) with λ_{max} at about 620, 530, 450 and 375 nm.[20-22] A similar complement of cone pigments is found in salmonids such as trout,[23,24] in poecilids such as guppies[25] and in marine pomacentrids.[26] These species have the potential for tetrachromatic colour vision and, at least in goldfish, there is behavioural evidence to support the hypothesis.[27]

Reptiles and birds

In some respects these two classes of vertebrates (at least lizards and birds) may be considered together, since, from a somewhat simplistic evolutionary standpoint, birds can be thought of as 'feathered reptiles'. All the recent evidence from avian retinas suggests that most diurnal birds have, in addition to rods, a class of LWS double cones and at least four spectral classes of single cone. Each class of cone is characterised by a specific oil droplet which, in the case of the LWS and MWS, is brightly coloured. As with teleosts, the four classes of cone have λ_{max} in the 'red', 'green', 'blue' and 'violet or ultraviolet' regions of the spectrum. All avian pigments are rhodopsins based on vitamin A_1 with λ_{max} close to 570, 505, 450 and 400–420 or about 370 nm.[28] Although there is no direct behavioural evidence in birds, the retinal complement of four classes of spectrally distinct single cones probably subserves a tetrachromatic colour vision system.[29]

The reptiles form a more diverse class than birds, in which crocodiles (Crocodilia), lizards (Squamata) and turtles (Chelonia) are widely separated phylogenetically. Nevertheless, representatives of all three groups have been shown to possess four spectral classes of cone. The crocodilian, *Alligator mississippiensis*, has two classes of single cone containing rhodopsins with λ_{max} at about 535 and 444 nm, and unequal double cones with a LWS principal member, λ_{max} at 566 nm, and a MWS accessory member with λ_{max} at about 503 nm.[30] It is possible that the two longer-wave pigments (λ_{max} at 566 and 535 nm) are both members of the same LWS class of cone pigments (see below), and there would appear to be no violet- or ultraviolet-sensitive cone class.

A number of species of *Anolis* lizards have also been shown to have four spectral classes of cone with visual pigments with λ_{max} at about 565, 495, 450 and 365 nm, though in *A. carolinensis* porphyropsins are present, displacing the λ_{max} of the LWS pigment to about 625 nm.[31–33] In turtles (e.g. *Pseudemys elegans*) the retina is somewhat similar to that in birds in that it contains rods, double cones and four spectrally distinct classes of single cone, each containing a characteristic oil droplet.[34] As in birds, both members of the double cones are LWS with the single cones containing, in freshwater turtles (e.g. *Geoclemys reevesii*), porphyropsins with λ_{max} at about 620, 540, 460 and 370 nm.[35]

The available data from representatives of teleosts, reptiles and birds that are either terrestrial or live in shallow water and are therefore exposed to the full daylight spectrum, indicate that the presence of four spectral classes of cone is not uncommon. In most species the cone pigments appear to fall into four clear spectral regions with, in a rhodopsin system, LWS cones having λ_{max} close to 560 nm, MWS cones with λ_{max} around 500 nm, short-wave sensitive (SWS) cones with λ_{max} between about 440 and 460 nm and VS/UVS cones with λ_{max} either between about 400–420 nm or about 360–380 nm. Although there is little or no behavioural evidence to

support the hypothesis, it seems reasonable to suggest that these species have evolved a tetrachromatic colour vision system.

Mammals

In contrast to the potential tetrachromacy of many 'lower' vertebrates, mammals are primarily dichromatic with only two spectrally distinct classes of single cone.[36] Generally these consist of a LWS cone with λ_{max} between about 500 and 565 nm and a SWS cone with λ_{max} shorter than about 450 nm. The SWS cone appears to have λ_{max} either in the violet at about 420–440 nm, as in primates,[37–39] or in the ultraviolet at about 365 nm, as in some rodents.[40,41] In addition to being reduced to a limited dichromacy, cones are generally scarce in the mammalian retina, in contrast to the high density of cones in many diurnal fish, reptiles and birds.

Primates

It is only in primates that a higher order of colour vision, trichromacy, is found amongst the mammals. At the separation of the Old and New World monkeys about 35 MYA, there appear to have been two different solutions to acquiring trichromacy. In New World monkeys the gene for the LWS cone pigment became polymorphic leading to trichromacy in some females,[42] whereas in Old World monkeys separate genes code for the LWS and MWS cone pigments.[43]

In both groups of New World monkeys, the callitrichids and the cebids, the gene coding for the LWS pigment is located on the X chromosome and exists in three allelic forms. In the callitrichids the alleles yield cone pigments with λ_{max} at about 543, 556 and 563 nm,[44–46] whereas in cebids the λ_{max} are at about 535, 550 and 563 nm.[42,47,48] Since males inherit only a single X chromosome they can express only a single cone pigment in the long-wave spectral region and are therefore dichromats, but may be one of three variants. Likewise, females homozygous at the long-wave opsin locus will also be dichromatic. However, because of X chromosome inactivation, females heterozygous at the locus will have the benefit of trichromacy.[44]

In Old World monkeys and the Great Apes including humans, the two genes on the X chromosomes demonstrate 98% identity at the DNA sequence level and are likely to have arisen from unequal recombination.[43,49,50] These two visual pigments with λ_{max} at about 563 and 535 nm represent variations within the same family of LWS opsins. A similar recombination even seems to have occurred more recently, about 15 MYA, in at least one genus of platyrrhine monkey, the howler monkey *Alouatta*, conferring trichromacy on all members of the genera, both male and female.[51]

There is therefore a marked difference between the potential tetrachromacy exhibited by some teleosts, reptiles and birds, the dichromacy of the majority of mammals and the relatively recent expression of trichromacy amongst some primates. Three questions

immediately come to mind. (i) Have the four classes of cone visual pigments present in 'lower' vertebrates evolved separately within each vertebrate class, or do they represent a basic ancestral condition present at a very early stage in vertebrate evolution? (ii) Why are mammals basically dichromatic? (iii) Why has trichromacy 're-evolved' within primates? The pioneering work of Nathans[43] in identifying and sequencing the opsin genes of the human cone visual pigments has led to the subsequent sequencing of a large number of opsin genes from a great variety of species including representatives from all the major vertebrate classes. From these gene sequences, it is possible to develop a phylogeny of opsins that indicates the molecular evolution of visual pigments within the vertebrates and suggests answers to some of the above questions.

Visual pigment phylogeny

A very abridged and simplified phylogenetic tree is given in Fig. 1, which is loosely based on that of Yokoyama.[52] It is not possible here to include all the sequenced opsins, but only to give the basic underlying pattern that is emerging. From the tree, it is clear that an early gene duplication, about 400 MYA ago, and subsequent mutations, led to the separation of a LWS group of opsins, the majority of which show anion sensitivity, from a SWS group that are anion insensitive. It is probable that the anion-insensitive group is
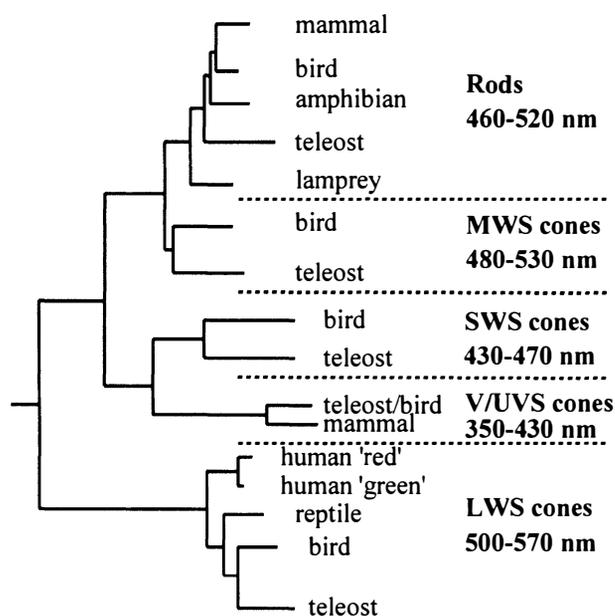

Fig. 1. *A phylogenetic tree of vertebrate visual pigments. The tree (greatly simplified from Yokoyama[52]), illustrates an early gene duplication to produce longer- and shorter-wave cone pigments. The initial division was rapidly followed by two further gene duplications to produce three spectral classes of cones within the shorter-wave family. A fourth gene duplication led to the evolution of rod pigments. Mammals retain cone pigments only from the longest- and shortest-wavelength cone pigment families. Gene duplication within the long-wave cone class has provided the basis for trichromacy in primates including humans.*

ancestral, since all invertebrate visual pigments are anion-insensitive. The existence of two spectral classes of cone would form the basis of a dichromatic colour vision system. Two more gene duplications within the SWS opsin group led to three opsin classes giving cone visual pigments with λ_{max} in the regions of about 360–450, 440–470 and 470–520 nm. A fourth gene duplication within the MWS opsins led to the rod opsins, also with λ_{max} around 500 nm. The five classes of opsins show about a 40% identity with each other, whereas the identity within each family is better than 80%.

All these gene duplications appear to have occurred very early in vertebrate evolution, about 350 MYA, ancestral to all the major present-day classes of vertebrate. The earliest visual pigments were cone pigments, with rod opsins evolving last. The potential tetrachromacy found in many teleosts, reptiles and birds therefore has a common origin and has not evolved separately within each class.

The evidence from the phylogenetic tree suggests that mammals have retained only two of the four classes of cone opsin: the shortest- and longest-wavelength classes. One assumption that may explain the loss of the middle- and short-wave opsin classes is that during the early evolution of mammals from their reptilian ancestors there was a nocturnal phase during which complex tetrachromatic colour vision was lost. As nocturnal species, the pro-mammals increased their sensitivity to low light levels with a concomitant reduction of colour vision to a minimal dichromatic system. Indeed, in some present-day nocturnal mammals the photopic system has been further reduced to monochromacy based only on the longer-wave pigment.[53]

The emergence of trichromacy within the primates (or at least the Old World monkeys) may be correlated with the evolution of specific yellow/orange fruits in tropical rain forests. The consequence of the dichromacy of most mammals is that they are red/green colour blind, in terms of human colour perception. However, if the visual task is to detect ripe (yellow/orange) fruit against a 'green' background of leaves varying greatly in luminance, then the task is made far easier by using chromaticity rather than luminance. In other words, their colour vision requires two spectral classes of cone maximally sensitive in the red/green spectral region.[54–59]

Since the LWS and MWS cone opsins in Old World primates including humans are about 98% identical at the amino acid level, the spectral difference between them of 25–30 nm must be due to the remaining 2% difference. Of these 14 amino acids, seven appear to have spectral effects, but only three seem to be primarily involved in the spectral separation.[43,60–62] The sites are 164 (mammalian rod opsin numbering) in helix IV and sites 261 and 269 in helix VI. All three sites face towards the retinal binding pocket and can directly affect the charge distribution around retinal. In the MWS pigment the three sites are occupied by non-hydroxyl-bearing amino acids – alanine, phenylalanine and alanine respectively – whereas in the LWS pigment these amino acids are replaced by their polar, hydroxyl-bearing

analogues – serine, tyrosine and threonine. The two sites in helix VI account for the major spectral displacement, with site 164 contributing only about 2–6 nm. Alternative combinations of amino acids at the three sites account for the polymorphic pigments amongst the New World monkeys[45,63] and for the anomalous pigments in human colour deficiencies.[64–66]

The recent gene duplication and mutations that have given rise to the series of longer-wave pigments in primates also appear to have occurred independently in some teleosts[67] and probably within some reptiles.[30] These examples of convergent evolution suggest that there are relatively few amino acid sites in the LWS opsin class where substitutions can occur to spectrally tune visual pigments and maintain their function. Similarly, it seems that the division of the violet/ultraviolet class of opsins into spectrally distinct pigments with λ_{max} above 400 nm and λ_{max} below about 380 nm has also occurred independently within teleosts, birds and mammals.[68–70]

In addition to the opsins that produce *visual* pigments, that is pigments located in retinal rods and cones and which are used in image perception, there are other classes of opsin, both ocular and extraocular, that are not directly involved in vision. Perphaps the most notable of these is an opsin, P-opsin or pinopsin, expressed in the pineal of reptiles and birds.[71–74] This opsin forms a separate and ancient phylogenetic class of photosensitive pigments probably dividing from the visual pigment opsins just prior to or just after the first visual pigment opsin gene duplication separating the LWS opsins from the SWS opsins.

At least two additional opsin classes have been identified that are expressed in the retina. One, retinal G protein-coupled receptor or RGR, has been located in mammals and is expressed in Müller cells and the retinal pigment epithelium. It preferentially binds *all-trans* retinal to produce a blue/ultraviolet pigment and is thought to play a role in the visual process.[75-77] More recently, a further opsin family, salopsin (first described from salmon) or vertebrate ancient (VA) opsin, has been identified within the retina of teleosts,[78] which is totally separated from the visual pigment opsins and P-opsins. VA opsin shows a 32–42% amino acid identity with the known visual pigment opsins and phylogenetic analysis suggests that this opsin diverged early in the evolution of vertebrate photopigments and may well precede the initial visual pigment gene duplication.

References

1. Crescitelli F. The visual cells and visual pigments of the vertebrate eye. In: Dartnall HJA. editor. Photochemistry of vision, vol VII/1. Berlin: Springer, 1972:245–363.
2. Govardovskii VI, Lychakov DV. Visual cells and visual pigments of the lamprey, *Lampetra fluviatilis*. J Comp Physiol [A] 1984;154:279–86.
3. Ishikawa M, Takao M, Washioka H, Tokunaga F, Watanabe H, Tonosaki A. Demonstration of rod and cone photoreceptors in the lamprey retina by freeze replication and immunofluorescence. Cell Tissue Res 1987;249:241–6.
4. Negishi K, Teranishi T, Kuo C-H, Miki N. Two types of lamprey retina photoreceptors immunoreactive to rod- or cone-specific antibodies. Vision Res 1987;27:1237–41.
5. Hisatomi O, Iwasa T, Tokunaga F. The visual pigment of lamprey. In: Hara T, editor. Molecular physiology of retinal proteins. Osaka: Yamada Science Foundation, 1988:371–2.
6. Hisatomi O, Iwasa T, Tokunaga F, Yasui A. Isolation and characterization of lamprey rhodopsin cDNA. Biochem Biophys Res Commun 1991;174:1125–32.
7. Zhang H, Yokoyama S. Molecular evolution of the rhodopsin gene of marine lamprey, *Petromyzon marinus*. Gene 1997;191:1–6.
8. Hárosi FI, Kleinschmidt J. Visual pigments in the sea lamprey, *Petromyzon marinus*. Vis Neurosci 1993;10:711–5.
9. Knowles A. The effects of chloride ions upon chicken visual pigments. Biochem Biophys Res Commun 1976;73:56–62.
10. Crescitelli F. Ionochromic behavior of gecko visual pigments. Science 1977;195:187–8.
11. Novitsky IY, Zak PP, Ostrovsky MA. The effects of anions on absorption spectrum of the longwavelength retinal-containing pigment iodopsin in native frog cones (a microspectrophotometric study). Bioorg Chem 1989;15:1037–43.
12. Kleinschmidt J, Hárosi FI. Anion sensitivity and spectral tuning of cone visual pigments *in situ*. Proc Natl Acad Sci USA 1992;89:9181–5.
13. Wang Z, Asenjo AB, Oprian DD. Identification of the Cl binding site in the human red and green color vision pigments. Biochemistry 1993;32:2125–30.
14. Sun H, Macke JP, Nathans J. Mechanisms of spectral tuning in the mouse green cone pigment. Proc Natl Acad Sci USA 1997;94:8860–5.
15. Govardovskii VI, Byzov AL, Zueva LV, Polisczuk NA, Baburina EA. Spectral characteristics of photoreceptors and horizontal cells in the retina of the Siberian sturgeon *Acipenser baieri* Brandt. Vision Res 1991;31:2047–56.
16. Govardovskii VI, Röhlich P, Szél A, Zueva LV. Immunocytochemical reactivity of rod and cone visual pigments in the sturgeon retina. Vis Neurosci 1992;8:531–7.
17. Loew ER, Sillman AJ. Age-related changes in the visual pigments of the white sturgeon (*Acipenser transmontanus*). Can J Zool 1993;71:1552–7.
18. Sillman AJ, Sorsky ME, Loew ER. The visual pigments of wild white sturgeon (*Acipenser transmontanus*). Can J Zool 1995;73:805–9.
19. Bowmaker JK. The visual pigments of fish. In: Osborne NN, Chader GJ, editors. Progress in retinal and eye research, vol 15. Oxford: Pergamon Press, 1995:1–31.
20. Avery JA, Bowmaker JK, Djamgoz MBA, Downing JEG. Ultraviolet sensitive receptors in a freshwater fish. J Physiol (Lond) 1983;334:23P.
21. Hárosi FI, Hashimoto Y. Ultraviolet visual pigment in a vertebrate: a tetrachromatic cone system in the dace. Science 1983;222:1021–3.
22. Bowmaker JK, Thorpe A, Douglas RH. Ultraviolet-sensitive cones in the goldfish. Vision Res 1991;31:349–52.
23. Bowmaker JK, Kunz YW. Ultraviolet receptors, tetrachromatic colour vision and retinal mosiacs in the brown trout (*Salmo trutta*): age dependent change. Vision Res 1987;27:2101–8.
24. Hawryshyn CW, Hárosi FI. Spectral characteristics of visual pigments in rainbow trout (*Oncorhynchus mykiss*). Vision Res 1994;34:1385–92.
25. Archer SN, Lythgoe JN. The visual pigment basis for cone polymorphism in the guppy, *Poecilia reticulata*. Vision Res 1990;30:225–33.
26. McFarland WN, Loew ER. Ultraviolet visual pigments in marine fishes of the family Pomacentridae. Vision Res 1994;34:1393–6.

27. Neumeyer C. Tetrachromatic color vision in goldfish: evidence from color mixture experiments. J Comp Physiol [A] 1992;171:639–49.

28. Bowmaker JK, Heath LA, Wilkie SE, Hunt DM. Visual pigments and oil droplets from six classes of photoreceptor in the retinas of birds. Vision Res 1997;37:2183–94.

29. Maier EJ, Bowmaker JK. Colour vision in a passeriform bird, *Leiothrix lutea*: correlation of visual pigment absorbance and oil droplet transmission with spectral sensitivity. J Comp Physiol [A] 1993;172:295–301.

30. Sillman AJ, Ronan SJ, Loew ER. Histology and microspectrophotometry of the photoreceptors of a crocodilian, *Alligator mississippiensis*. Proc R Soc Lond B 1991;243:93–8.

31. Fleishman LJ, Loew ER, Leal M. Ultraviolet vision in lizards. Nature 1993;365:397.

32. Provencio I, Loew ER, Foster RG. Vitamin A_2-based visual pigments in fully terrestrial vertebrates. Vision Res 1992;32:2201–8.

33. Kawamura S, Yokoyama S. Functional characterization of visual and nonvisual pigments of American chameleon (*Anolis carolinensis*). Vision Res 1998;38:37–44.

34. Goede P, Kolb H. Identification of the synaptic pedicles belonging to the different spectral types of photoreceptor in the turtle retina. Vision Res 1994;34:2801–11.

35. Ohtsuka T. Spectral sensitivities of seven morphological types of photoreceptors in the retina of the turtle, *Geoclemys reevesii*. J Comp Neurol 1985;237:145–54.

36. Jacobs GH. The distribution and nature of colour vision among the mammals. Biol Rev 1993;68:413–71.

37. Bowmaker JK, Dartnall HJ. Visual pigments of rods and cones in a human retina. J Physiol (Lond) 1980;298:501–11.

38. Bowmaker JK, Dartnall HJ, Mollon JD. Microspectrophotometric demonstration of four classes of photoreceptor in an old world primate, *Macaca fascicularis*. J Physiol (Lond) 1980;298:131–43.

39. Bowmaker JK, Astell S, Hunt DM, Mollon JD. Photosensitive and photostable pigments in the retinae of Old World monkeys. J Exp Biol 1991;156:1–19.

40. Jacobs GH, Neitz J, Deegan JF. Retinal receptors in rodents maximally sensitive to ultraviolet light. Nature 1991;353:655–6.

41. Jacobs GH, Deegan JF. Sensitivity to ultraviolet light in the gerbil (*Meriones unguiculatus*): characteristics and mechanisms. Vision Res 1994;34:1433–41.

42. Mollon JD, Bowmaker JK, Jacobs GH. Variations of colour vision in a New World primate can be explained by polymorphism of retinal photopigments. Proc R Soc Lond B 1984;222:373–99.

43. Nathans J, Thomas D, Hogness DS. Molecular genetics of human color vision: the genes encoding blue, green and red pigments. Science 1986;232:193–203.

44. Tovée MJ, Bowmaker JK, Mollon JD. The relationship between cone pigments and behavioural sensitivity in a New World monkey (*Callithrix jacchus jacchus*). Vision Res 1992;32:867–78.

45. Williams AJ, Hunt DM, Bowmaker JK, Mollon JD. The polymorphic photopigments of the marmoset: spectral tuning and genetic basis. EMBO J 1992;11:2039–45.

46. Jacobs GH, Neitz J, Crognale MA. Color vision polymorphism and its photopigment basis in a callitrichid monkey (*Saguinus fusicollis*). Vision Res 1987;27:2089–100.

47. Jacobs GH, Neitz J. Color vision in squirrel monkeys: sex-related differences suggest a mode of inheritance. Vision Res 1985;25:141–3.

48. Shyue SK, Hewett Emmett D, Sperling HG, Hunt DM, Bowmaker JK, Mollon JD, Li WH. Adaptive evolution of color vision genes in higher primates. Science 1995;269:1265–7.

49. Vollrath D, Nathans J, Davis RW. Tandem array of human visual pigment genes at Xq28. Science 1988;240:1669–72.

50. Dulai KS, Bowmaker JK, Mollon JD, Hunt DM. Sequence divergence, polymorphism and evolution of the middle-wave and long-wave visual pigment genes of Great Apes and Old World monkeys. Vision Res 1994;34:2483–91.

51. Jacobs GH, Neitz M, Deegan JF, Neitz J. Trichromatic color vision in New World monkeys. Nature 1996;382:156–8.

52. Yokoyama S. Gene duplications and evolution of the short wavelength-sensitive visual pigments in vertebrates. Mol Biol Evol 1994;11:32–9.

53. Jacobs GH, Neitz M, Neitz J. Mutations in S-cone pigment genes and the absence of color vision in two species of nocturnal primate. Proc R Soc Lond B 1996;263:705–10.

54. Mollon JD. 'Tho' she kneel'd in that place where they grew . . . '. J Exp Biol 1989;146:21–38.

55. Nagle MG, Osorio D. The tuning of human photopigments may minimize red–green chromatic signals in natural conditions. Proc R Soc Lond B 1993;252:209–13.

56. Julliot C. Seed dispersal by red howling monkeys (*Alouatta seniculus*) in the tropical rain forest of French Guiana. Int J Primatol 1996;17:239–58.

57. Osorio D, Vorobyev M. Color vision as an adaptation to frugivory in primates. Proc R Soc Lond B 1996;263:593–9.

58. Regan BC, Vienot F, Charlesdominique PC, Pefferkorn S, Simmen B, Julliot C, Mollon JD. The color signals that fruits present to primates. Invest Ophthalmol Vis Sci 1996;37:2997.

59. Savage A, Dronzek LA, Snowdon CT. Color discimination by the cotton-top tamarin (*Saguinus oedipus oedipus*) and its relation to fruit coloration. Folia Primatol (Basel) 1987;49:57–69.

60. Asenjo AB, Rim J, Oprian DD. Molecular determinants of human red/green colour discrimination. Neuron 1994;12:1131–8.

61. Oprian DD, Asenjo AB, Lee N, Pelletier SL. Design, chemical synthesis, and expression of genes for the three human color vision pigments. Biochemistry 1991;30:11367–72.

62. Merbs SL, Nathans J. Role of hydroxyl-bearing amino acids in differentially tuning the absorption spectra of the human red and green cone pigments. Photochem Photobiol 1993;58:706–10.

63. Neitz M, Neitz J, Jacobs GH. Spectral tuning of pigments underlying red–green color vision. Science 1991;252:971–4.

64. Neitz J, Neitz M, Kainz PM. Visual pigment gene structure and the severity of color vision defects. Science 1996;274:801–4.

65. Sanocki E, Teller DY, Deeb SS. Rayleigh match ranges of red/green color-deficient observers: psychophysical and molecular studies. Vision Res 1997;37:1897–907.

66. Deeb SS, Motulsky AG. Molecular genetics of human color vision. Behav Genet 1996;26:195–207.

67. Yokoyama R, Yokoyama S. Convergent evolution of the red-and green-like visual pigment genes in fish, *Astyanax fasciatus*, and human. Proc Natl Acad Sci USA 1990;87:9315–8.

68. Chiu MI, Zack DJ, Wang YS, Nathans J. Murine and bovine blue cone pigment genes: cloning and characterization of two new members of the S family of visual pigments. Genomics 1994;21:440–3.

69. Hisatomi O, Satoh T, Barthel LK, Stenkamp DL, Raymond PA, Tokunaga F. Molecular cloning and characterization of the putative ultraviolet-sensitive visual pigment of goldfish. Vision Res 1996;36:933–9.

70. Wilkie SE, Vissers PMAM, Das D, de Grip WJ, Bowmaker JK, Hunt DM. UV vision in birds: spectral characteristics, cDNA sequence and retinal localisation of the UV-sensitive pigment of the budgerigar. Biochem J 1998;330:541–7.

71. Max M, McKinnon PJ, Seidenman KJ, Barrett RK, Applebury ML, Takahashi JS, Margolskee RF. Pineal opsin: a nonvisual opsin expressed in chick pineal. Science 1995;267:1502–6.

72. Okano T, Yoshizawa T, Fukada Y. Pinopsin is a chicken pineal photoreceptive molecule. Nature 1994;372:94–7.

73. Kawamura S, Yokoyama S. Expression of visual and nonvisual opsins in American chameleon. Vision Res 1997;37:1867–71.

74. Kawamura S, Yokoyama S. Molecular characterization of the pigeon P-opsin gene. Gene 1996;182:213–4.

75. Shen D, Jiang M, Hao W, Tao L, Salazar M, Fong HK. A human opsin-related gene that encodes a retinaldehyde-binding protein. Biochemistry 1994;33:13117–25.

76. Hao W, Fong HK. Blue and ultraviolet light-absorbing opsin from the retinal pigment epithelium. Biochemistry 1996;35:6251–6.

77. Pandey S, Blanks JC, Spee C, Jiang M, Fong HK. Cytoplasmic retinal localization of an evolutionary homolog of the visual pigments. Exp Eye Res 1994;58:605–13.

78. Soni BG, Foster RG. A novel and ancient vertebrate opsin. FEBS Lett 1997;406:279–83.