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PERSPECTIVE

Primate color vision: A comparative perspective

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Abstract

Thirty years ago virtually everything known about primate color vision derived from psychophysical studies of normal and color-defective humans and from physiological investigations of the visual system of the macaque monkey, the most popular of human surrogates for this purpose. The years since have witnessed much progress toward the goal of understanding this remarkable feature of primate vision. Among many advances, investigations focused on naturally occurring variations in color vision in a wide range of nonhuman primate species have proven to be particularly valuable. Results from such studies have been central to our expanding understanding of the interrelationships between opsin genes, cone photopigments, neural organization, and color vision. This work is also yielding valuable insights into the evolution of color vision.

Keywords: Cone photopigments, Opsin genes, Evolution, Retinal organization, Primate color vision

Introduction

In his classical review of vertebrate eyes, Gordon Walls drew a sharp distinction between the apparently limited color vision capacities of most mammals and those enjoyed by primates by remarking, "... we come at last to the primate order. Here ... there has never been any doubt of the occurrence of color vision in all its glory." (Walls, 1942). Having said that, however, he then went on to list evidence to suggest that even among the various primate lineages, color vision is probably not an invariant capacity. The species variations hinted in Walls' early review turned out to be more profound than he imagined, and in recent years studies of these variations have added important detail to the growing catalogue of perceptual capacities in various primates. Equally important, this work has also proven valuable in advancing our general understanding of primate color vision in at least three ways: (1) by providing deep insights into the early stage biological mechanisms that support color vision, (2) by fostering a data-based scenario for how color vision may have evolved, and (3) by setting the stage for a greatly renewed interest in the ecology of primate color vision. Here, I review and comment on the first two of these topics, the third having recently been addressed in detail in several publications (Regan et al., 2001; Dominy, 2004; Osorio et al., 2004; Vorobyev, 2004; Jacobs, 2007).

Vertebrate photopigments and their evolution

> We have seen that parts many times repeated are eminently liable to vary in number and structure; consequently it is quite probable that natural selection, during the long-continued course of modification, should have seized on a certain number of primordially similar elements, many times repeated, and have adapted them to diverse purposes.
>
> Charles Darwin (1859)

Some 45 years ago, application of the newly devised technique of microspectrophotometry yielded the first direct measurements of the absorption properties of the three types of cone photopigment of the primate retina (Brown & Wald, 1963; Marks et al., 1964). For all but the hardiest iconoclasts those measurements finally brought to close long-held arguments about the identity of the first-stage mechanisms underlying primate trichromacy. Through the exploitation of direct measurements of the absorption spectra of cone photopigments, and from inferences about the absorption properties of these pigments derived from the structure of opsin genes, recent years have witnessed an explosion of information about the spectra of cone photopigments in a wide range of vertebrate species. In turn, that information allows the construction of scenarios for the evolution of vertebrate photopigments.

The consensus view is that all vertebrate visual pigments are products of five families of opsin genes (for extensive reviews with specification of genes and photopigments for a large number of vertebrate species, see the following: Yokoyama, 2000; Hart & Hunt, 2007; Bowmaker, 2008). One family (termed Rh1) specifies rod opsins; the expression products of the others (SWS1, SWS2, Rh2, and LWS) are cone opsins. These groups are believed to have arisen through a series of gene duplications. The dating of these duplication events remains uncertain, but it seems likely that (a) the four cone opsin gene families have an ancient origin, probably having emerged as long as 540 million years ago (mya), and (b) rod photopigments appeared only following the divergence of

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the several cone opsin gene families (Collin & Trezise, 2004). Variations in gene sequence within each of these families yields photopigments that are selectively tuned to absorb maximally in different portions of the spectrum. Fig. 1 shows the range of peak sensitivities (λ_{max}) for photopigments derived from each of the five gene families. In some vertebrates, the absorption properties of the photoreceptors are further modified by variations in the nature of the chromophore and/or by intraocular filtering. The effects of the latter are particularly dramatic in those photoreceptors that incorporate colored oil droplets; in such cases, as found for instance in the eyes of some birds, reptiles, fishes, and amphibians, the effective peak sensitivity of the receptor may be shifted as much as 60 nm longer than the λ_{max} of the photopigment.

Of the major vertebrate groups, pigments derived from all four of the cone opsin gene families are found in many birds, fishes, and reptiles. Rh2 opsin genes appear to be absent from contemporary amphibians, while eutherian mammals have neither Rh2 opsin genes nor SWS2 opsin genes (Bowmaker, 2008). The timing and circumstances of the loss of these two are not known, although it is usually assumed to have occurred early in mammalian evolution, likely coincident with the nocturnal phase of mammalian history. It has recently been discovered that species from Marsupalia and Monotremata may constitute exceptions to this mammalian principle in that three functional classes of cone have been detected in some Australian marsupials, one an SWS1 gene, one an LWS gene, and one that may be an Rh2 gene (Arrese et al., 2002, 2006), while the monotreme platypus appears to have two functional cone opsin genes (drawn, respectively, from the LWS and SWS2 gene families) plus an additional, but nonfunctional, SWS1 opsin gene (Davies et al., 2007).

For primates, the important functional consequence of this account of the evolution of vertebrate opsin genes is that the photopigments available to support color vision are limited to representatives drawn from only two of the cone opsin gene families, LWS and SWS1 (Fig. 1).

Evolution of primate cone pigments

As for vertebrates in general, the picture of the evolution of cone photopigments among the primates has been derived from study of contemporary species, from inferences gleaned from examination of fossil material, and from molecular comparisons of cone opsin genes.

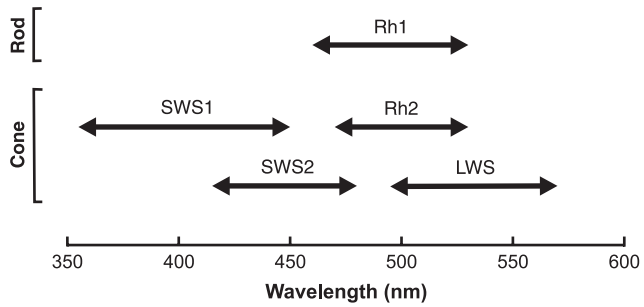

Fig. 1. Five opsin gene families specify all the photoreceptor opsins found in vertebrate retinas. One of these (Rh1) is associated with photopigments found in rods; the other four link to cone pigments. The extents of the horizontal lines indicate the total range of λ_{max} values for retinal 1–based pigments specified by each of the five gene families.

Primate phylogeny

Fig. 2 is a simplified primate phylogeny that provides a convenient framework for the subsequent discussion of the evolution of cone photopigments. It represents results from a molecular comparison of sequences derived from autosomal genes obtained from 13 species of extant primates (Steiper & Young, 2006). The number of living primates (~350 species) vastly exceeds this sample size, but the animals examined can be considered as representative for present purposes. The timings of the divergence events in phylogenies such as those of Fig. 2 are matters of continuing debate, subject to reinterpretation as new evidences emerge. Generally, analyses of the fossil record lead to more recent divergence estimates than do phylogenies derived from molecular comparisons. For example, the earliest available fossil material for euprimates (''primates of modern aspect'') dates to ~55 mya, whereas molecular-based estimates, such as those of Fig. 2, often place their origin much earlier—in the Cretaceous, perhaps ~80 mya. A recent statistical analysis designed to account for gaps in the fossil record provides support for the earlier date for the origin of primates (Martin et al., 2007), as does a species-level reconstruction of a phylogeny for nearly all the extant species of mammals (Bininda-Emonds et al., 2007).

The earliest primates: Nocturnal or diurnal?

Although the exact timing of the events characterizing primate evolution is not critical for the current understanding of how primate color vision may have evolved, it is relevant to ask about the photic habits of the earliest representatives of our order. Based principally on an analysis of their sizes, the traditional interpretation is that for much of their early history, mammals were small and nocturnal (Kielan-Jaworowska et al., 2004). Starting from that understanding and buttressed by a series of large-scale comparisons of a variety of physical features of fossil and contemporary primate material, it has been widely assumed that ancestral primates were themselves nocturnal (Martin, 1990; Heesy & Ross, 2001; Martin & Ross, 2005). In support of this idea, a recent examination of craniodental remains from a basal African anthropoid dated ~37 mya indicates that it had orbital features consistent with a nocturnal lifestyle (Seiffert et al., 2005) If early primates were nocturnal, as these observations would suggest, it would seem logical that the cone pigment complements of these earliest primates were those now typical of most mammals, all of which had a nocturnal past, that is, two types of cone photopigment, each drawn, respectively, from the SWS1 and LWS opsin gene families (Fig. 1). The spectral positioning of SWS1-based pigment in early primates is uncertain, but from a comparative analysis of LWS opsin genes from a number of mammals, it has been suggested that these animals may have had an LWS pigment with λ_{max} of ~553 nm (Yokoyama & Radlwimmer, 1999).

There are challenges to the view that the earliest primates were nocturnal. For example, examination of a number of features of a skull from a euprimate dated 55 mya has been taken to suggest that this animal was small and diurnal (Ni et al., 2003). And a comparative examination of cone opsin genes from a variety of strepsirrhine species has also been interpreted as consistent with the idea that the ancestral primates were not nocturnal (Tan et al., 2005). Both of these claims have been refuted (Ross & Martin, 2007) and for now the weight of evidence seems firmly on the side of those who believe that our earliest primate ancestors were nocturnal.

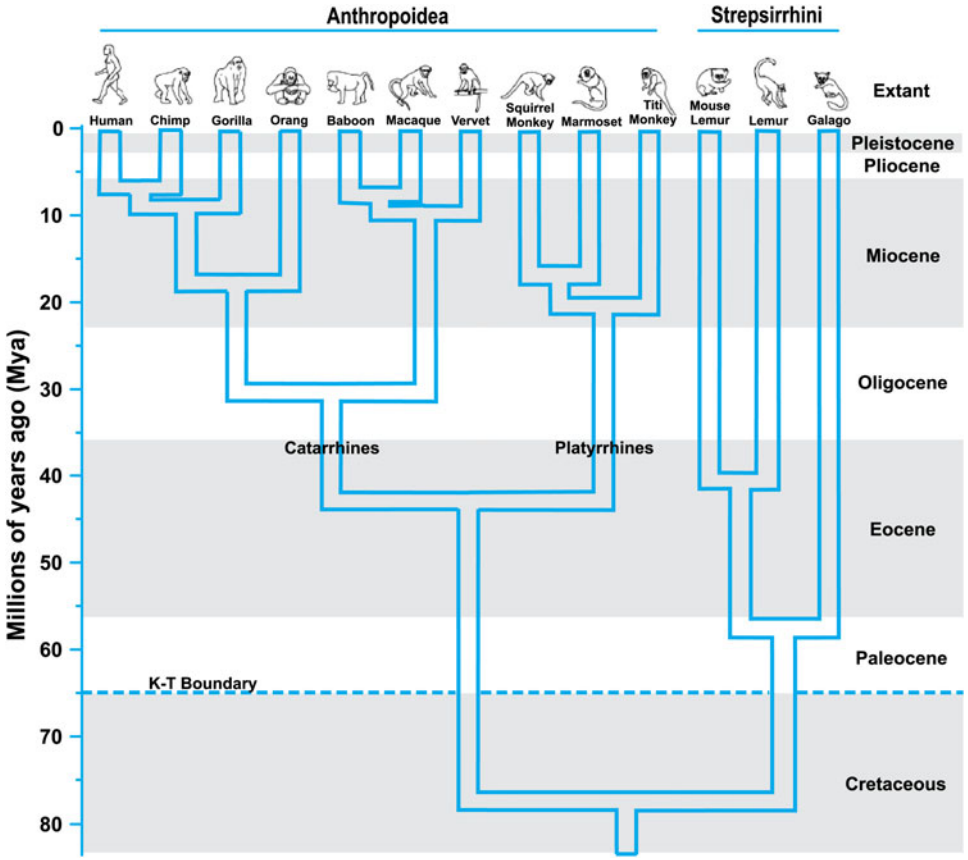

Fig. 2. Phylogeny of the major primate groups. The divergence times were estimated from a Bayesian analysis of genomic data obtained from 13 species of extant primates. So derived, the last common ancestor of modern primates dates to ~77 mya, while the two major groups of anthropoids, platyrrhines (New World monkeys) and catarrhines (Old World monkeys, apes, and humans), diverged ~43 mya. Modified from Steiper and Young (2006).

Evolution and nature of catarrhine cone pigments

Unlike most mammals, representative catarrhine species (a group made up of Old World monkeys, apes, and humans; Fig. 2) have two X-chromosome cone opsin genes. These genes are located adjacent to one another on the X-chromosome and are highly homologous, indicating that they probably emerged as a result of a recent gene duplication (Nathans et al., 1986). The fact that the catarrhine gene arrangement is distinctive from that characteristic of the platyrrhine (New World) monkeys (below), and that all catarrhines seem to share the same arrangement, implies that this duplication occurred following the platyrrhine–catarrhine divergence, but prior to the diversification of the catarrhines, at perhaps ~35 mya (Fig. 2). Although there are numerous catarrhine species that have not been studied, those that have (in total, animals drawn from at least 10 genera) all seem to have separate classes of cone containing M (middle-wavelength sensitive) and L (long-wavelength sensitive) pigments with respective λ_{max} values of ~530 and 560 nm (Bowmaker et al., 1991; Jacobs, 1996; Jacobs & Deegan, 1999).

The retinas of all catarrhines also have a small population of photoreceptors (some 5%–10% of all cones—Calkins, 2001) containing S (short-wavelength sensitive) pigment. The opsin gene specifying this pigment is localized to chromosome 7. The current view is that the ancestral mammalian SWS1 pigment was maximally sensitive in the ultraviolet (UV). In primates, as in many other mammalian lineages, a combination of substitutions in the opsin sequence shifted the spectral positioning of the pigment such that it absorbs maximally in the short-wavelength portion of the visible spectrum (Hunt et al., 2004). When that event may have occurred is not known. There are relatively few direct measurements of the absorption properties of primate S pigments, but there is reason to believe that, at least, there may be a modest difference between the human S pigment and that for various catarrhine monkeys with the human pigment being positioned relatively shorter (respective λ_{max} values of ~430 and 415 nm) (Bowmaker, 1990). Absorption spectra for the nominal three types of cone pigments found in catarrhine primates are sketched in Fig. 3A.

Catarrhines effectively share their opsin gene and cone photopigment complements, but there are several known species idiosyncrasies, and there are probably more yet to be discovered. The most prominent of these is the presence of gene/pigment polymorphisms in humans that are effectively absent in other catarrhines. The human polymorphisms encompass both the large-scale changes collectively linked to color-defective vision and a number of alterations that seemingly have little impact on normal vision (Neitz & Neitz, 2003). The former include the absence of one or the other of the normal M or L pigment (resulting in dichromatic color vision) and significant shifts in the spectral positioning of one of the M or L pigment (yielding anomalous trichromacies). Together, these polymorphisms affect ~8% of all Caucasian males. Though they have been less well

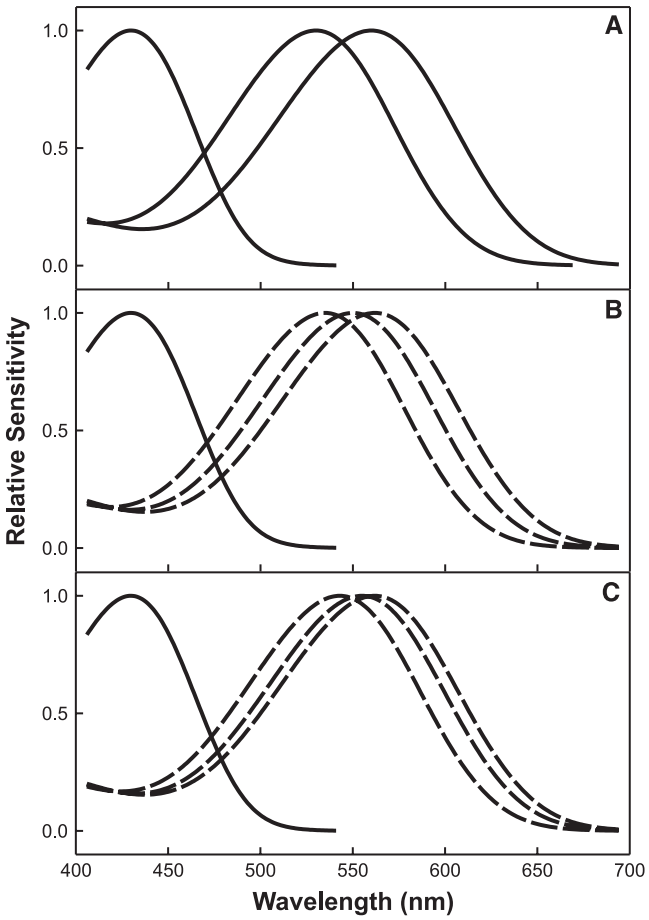

Fig. 3. Spectral sensitivity functions for primate cone photopigments. (**A**) Three types of cone pigment characteristic of the catarrhine primates. (**B**) Cone pigments of platyrrhine monkeys from the family Cebidae. (**C**) Cone pigments of platyrrhine monkeys from the family Callitrichidae. Pigments sketched as solid lines are those characteristically found in all individuals; the polymorphic M/L pigments appear as dashed lines. The λ_{max} values for all these cone pigments are given in the text.

scrutinized, analogous changes are, at best, infrequent in other catarrhines. That fact is made clear in Table 1, which documents that the incidence of dichromacy in a large population of human males is strikingly higher than that in a collection of male macaque monkeys. Although a number of suggestions have been offered as to the origin of this dramatic difference between these lineages, including a well-known early proposal that it may have resulted from a relaxation of selection against defective color vision in modern humans (Post, 1962), there is as yet no agreed explanation for this difference (Jacobs & Williams, 2001).

Table 1. *Assessments of the incidence of dichromatic color vision in two primate populations*

Population	Sample size (*n*)	Prevalence (%)
Human males	20,836	2.56
Macaque males	1629	0.002

The human results were drawn from several large surveys conducted on Caucasian males that are cited in Fletcher and Voke (1985). Results for the macaque monkeys were derived from Jacobs and Williams (2001) and Onishi et al. (1999).

Evolution and nature of platyrrhine cone pigments

> A case, of sorts, could be made for considering that trichromacy has evolved independently in the catarrhines and platyrrhines.
>
> Gordon L. Walls (1942)

As for the catarrhines, many platyrrhine lineages (Fig. 2) have succeeded in moving beyond the mammalian norm of a single type of M/L cone pigment. In the case of these monkeys, however, the additional pigments generally derive not from a gene duplication event, as they do in the catarrhines primates, but rather from the emergence of opsin gene polymorphisms (for reviews, see Jacobs, 1998, 2007). These polymorphisms are at an X-chromosome opsin gene locus, thus allowing individual animals to have varying opsin gene and cone photopigment complements. The typical arrangement features three opsin gene alleles, each specifying an M/L pigment with a unique spectral absorption property. Individual monkeys have any one of the three and, along with the product of an S-cone gene (as for catarrhines, mapped to chromosome 7), their retinas contain a total of two types of cone pigment, or they have any pair of the M/L pigments which along with an S pigment gives them a total of three types of cone pigment. Since there is only a single opsin gene on any X-chromosome in these platyrrhines, to gain a total of three cone pigments requires that different genes be present on two X-chromosomes, that is, the occurrence of three photopigments is restricted to females who are heterozygous at the opsin gene site. The net result of this arrangement is that six different photopigment phenotypes are represented in these species.

Although most platyrrhine monkeys have opsin gene polymorphisms of the sort just described, they are not all the same. One source of variation is that different species have unique sets of M/L opsin genes, thus providing variations in the spectral absorption properties of the M/L pigments. Two patterns predominate. The three types of M/L pigments for species from the family Cebidae (Cebus and squirrel monkeys) feature λ_{max} values of ~535, 550, and 562 nm. By contrast, the three types of M/L pigments found in species of the family Callitrichidae (marmosets, tamarins, etc.) have pigments with λ_{max} values of ~543, 556, and 562 nm, respectively. Although they have been less intensively investigated, the cone pigments found in members of several other platyrrhine families tend to follow the latter pattern (a list of the platyrrhine pigments so far measured with accompanying discussion of the measurement details appears in Jacobs, 2007). Evidence also suggests that there are some variations in the number of polymorphic genes and pigments. For example, three genera (spider monkeys—*Ateles*, pygmy marmosets—*Cebuella*, and Goeldi monkeys—*Callimico*) appear to have two not three M/L pigment variants (Jacobs, 2007). This apparent reduction could be artifactual, reflective of nothing more than sampling problems, but if it is real this arrangement significantly limits the number and variety of pigment combinations present in these monkey populations. The other deviation of this type occurs in monkeys from the genus *Callicebus* (titi monkeys). These primates appear to have an embarrassment of pigment riches featuring a total of five alternative versions of the M/L pigments (Jacobs & Deegan, 2005), an arrangement that should also greatly affect the total number of available pigment combinations in the population.

As is true for the catarrhines, platyrrhine S-cone pigments have not yet been subject to extensive study. The available measurements suggest that platyrrhine S cone pigments have λ_{max} of

~430 nm with, possibly, some modest variation from this figure in different species. The absorption spectra for the two most prevalent patterns of cone pigments seen in the polymorphic platyrrhine monkeys are shown in Fig. 3B and 3C.

Two significant deviations from the polymorphic theme have been detected in platyrrhine monkeys. The first is in *Aotus*, the owl monkey. This monkey is unique in being the only anthropoid considered to be nocturnal. *Aotus* lacks the M/L opsin polymorphism, having a single type of M/L pigment (λ_{max} of ~543 nm). Even more intriguing, owl monkeys express no viable S-cone pigment with the result that their retina contains only a single cone type. The absence of S cones in *Aotus* results from the presence of mutational changes in the S-cone opsin gene that obviate protein expression (Jacobs et al., 1996*b*). The fact that this same change is found in several different species of *Aotus* implies that the loss of S cones must have appeared early in the history of this lineage (Levenson et al., 2007). Interestingly, in recent years, a loss of S cones having a similar genetic basis has also been documented in a number of other mammalian species (reviewed in Peichl, 2005). The mammals comprising this group occupy greatly disparate environmental niches and have varied evolutionary histories, making it uncertain as what event(s) may have precipitated these gene-driven losses of a cone type. Whatever the reason, the compelling functional consequence is that these mammals must lack any conventional color vision capacity.

A second deviation from the platyrrhine polymorphic pattern has been detected in monkeys from the genus *Alouatta* (howler monkeys). Surprisingly, these monkeys resemble the catarrhines in routinely having two X-chromosome opsin genes, so providing each individual with retinas containing separate populations of M and L cones (Jacobs et al., 1996*a*). The M and L cone pigments of howler monkeys have spectral absorption properties very similar to those of catarrhine primates, that is, λ_{max} values of ~530 and 560 nm (Saito et al., 2004).

The sequences for M/L opsin genes are very similar across all primates, for example, the M/L pigment genes for a catarrhine (the human) and two species of platyrrhine monkeys (squirrel and marmoset monkeys) share a sequence identity of 96% or greater (Neitz et al., 1991; Hunt et al., 1993). This high similarity in the face of variations in the spectral positioning of the M/L pigments indicates that only a small number of changes in gene structure must be responsible for variations in spectral tuning. In fact, it appears that most of the variation in spectral tuning over the primate M/L pigment range of ~30 nm is due to amino acid substitutions at only three sites in the opsin molecule (positions numbered 180, 277, and 285 in the diagram of Fig. 4). In each case, the replacement of a nonpolar amino acid with hydroxyl-bearing amino acid (or vice versa) yields a discrete shift in the peak of the pigment with the total shift of the pigment being effectively additive for changes at the three sites. The net effect of this control for spectral tuning is that the nonhuman primate M/L pigments occupy only a small number of spectral locations. Table 2 documents this fact for representative genera drawn from all three major groups of primate.

The conservatism of spectral tuning of the primate M/L pigments provides the basis for drawing inferences about the evolution of the platyrrhine cone pigments. Comparisons of the sequences of the M/L opsin genes in the platyrrhines suggest that the polymorphisms characteristic of these species are of long standing, extending well back into platyrrhine history (as much as

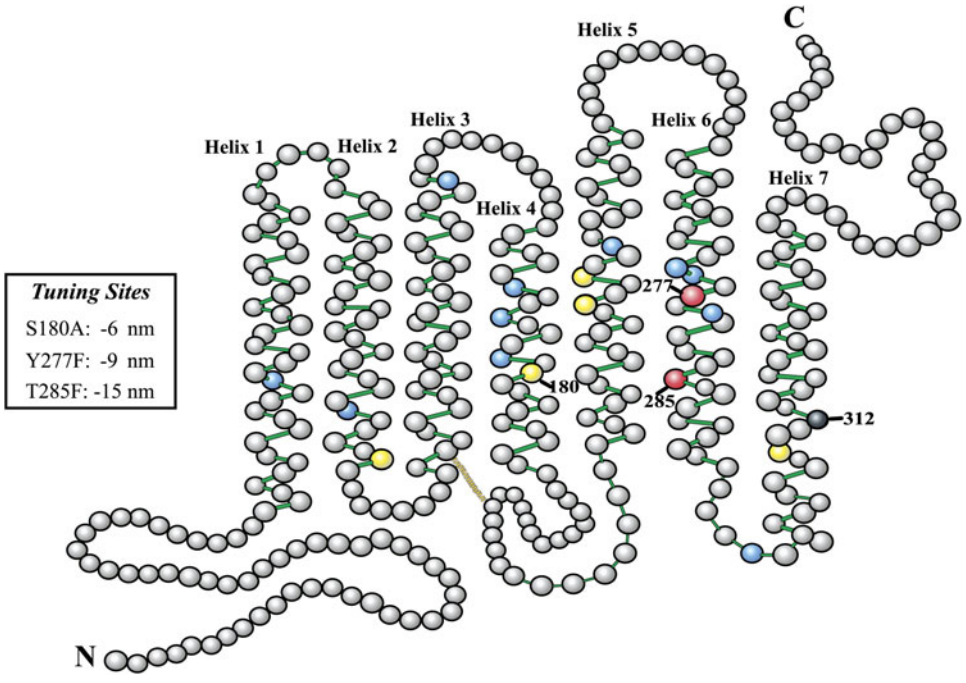

Fig. 4. Schematic representation of the primate M/L cone opsin. The amino acids are indicated by small circles. The opsin threads its way through the disc membrane of the cone outer segment in a series of seven linked helical arrays. The amino acids that are principally responsible for shifting the spectral absorption properties of the cone pigment are at the positions numbered 180, 277, and 285. Substitutions at those sites shift pigment absorption by approximately the amounts indicated in the inset; for example, substituting alanine for serine at position 180 shifts the pigment ~6 nm toward the shorter wavelengths. The retinal 1 chromophore is covalently bound to opsin at residue 312.

Table 2. *Amino acids involved in spectral tuning of M/L pigments in several genera of nonhuman primates*

Peak absorption (nm)	Genus*	Position 180	Position 277	Position 285
562	*Cebus*, *Saimiri*, *Callithrix*, *Saguinus*, *Alouatta*, *Macaca*	Serine	Tyrosine	Threonine
556	*Callithrix*, *Saguinus*, *Aotus*, *Propithecus*	Alanine	Tyrosine	Threonine
550	*Cebus*, *Saimiri*	Alanine	Phenylalanine	Threonine
543	*Callithrix*, *Saguinus*, *Propithecus*	Alanine	Tyrosine	Alanine
535	*Cebus*, *Saimiri*, *Alouatta*, *Macaca*	Alanine	Phenylalanine	Alanine

*Catarrhine: *Macaca*; platyrrhine: *Cebus*, *Saimiri*, *Callithrix*, *Saguinus*, *Alouatta*, *Aotus*. strepsirrhine: *Propithecus*.

20 mya), and that their evolution is better explained as having proceeded along lines more specific to the spectral positioning of the pigment rather than to strict species lineages (Boissinot et al., 1998; Surridge & Mundy, 2002). Howler monkeys with their two X-chromosome opsin gene sites obviously had a different history. As in the catarrhines, that must have included an X-chromosome opsin gene duplication. When the duplication may have emerged is uncertain, but the fact that it is unique to *Alouatta* means it occurred after this line had diverged from its sister clades, that is, more recently than ~12 mya (Schrago, 2007). Comparison of the M/L gene sequences in the howlers with those of catarrhine primates also strongly implies that the respective gene duplications were independent events, having occurred much more recently in the howler monkeys than in the catarrhines (Kainz et al., 1998).

Evolution and nature of strepsirrhine cone pigments

The suborder Strepsirrhini is composed of seven families of primates that are collectively native to Madagascar, Southeast Asia, and Africa. The eyes of the several dozen species that make up this diverse group of primates (Fig. 2) share a number of features that serve to sharply differentiate them from the eyes of anthropoids; among these, strepsirrhine retinas lack foveae and feature lowered cone:rod ratios, and their eyes often contain reflective tapeta. In the face of these generally more primitive features, these animals show a surprising range of cone photopigment arrangements. Although this story continues to develop, three general patterns have been detected to date. The first resembles that described for *Aotus*, that is, featuring a single functional pigment from the LWS gene family along with an S-cone opsin gene that has sufficient mutational changes to render it ineffective. All the lorises and bush babies appear to share this arrangement, along with a few species of lemur (Tan & Li, 1999; Kawamura & Kubotera, 2004; Tan et al., 2005). In a single case where it has been measured, the M/L cone pigment in such species has λ_{max} of ~543 nm (Jacobs et al., 1996*b*). Second, some strepsirrhines, ring-tailed and brown lemurs being common examples, have both functional S cones and a single type of M/L cone, an arrangement qualitatively similar to that of the mammalian norm (above). The spectral peaks of the pigments in animals of this type have an M/L pigment with a peak similar to those of the lorises and bush babies, that is, ~545 nm, while the S pigment peak in one measurement was at ~437 nm (Jacobs & Deegan, 1993). The third cone pigment pattern is generically similar to that seen in the polymorphic platyrrhines. Such animals have two alternative versions of M/L opsin gene and photopigments, along with an intact S-cone opsin gene (Tan & Li, 1999). This yields three different photopigment phenotypes to include heterozygous females whose retinas contain three separate cone types. The M and L cones of such animals have respective λ_{max} of ~545 and 558 nm, while S cones have peak values of ~430 nm (Jacobs et al., 2002). Individual species from two strepsirrhine families (Lemuridae and Indridae) have thus far been found to share this arrangement, but it probably also exists in additional species from these two families. These three varying patterns of strepsirrhine cone pigment arrangements are sketched in Fig. 5.

Among the strepsirrhines, the highly endangered aye-aye (*Daubentonia*) undoubtedly takes the prize as the most unusual. Examination of the opsin genes in this profoundly nocturnal

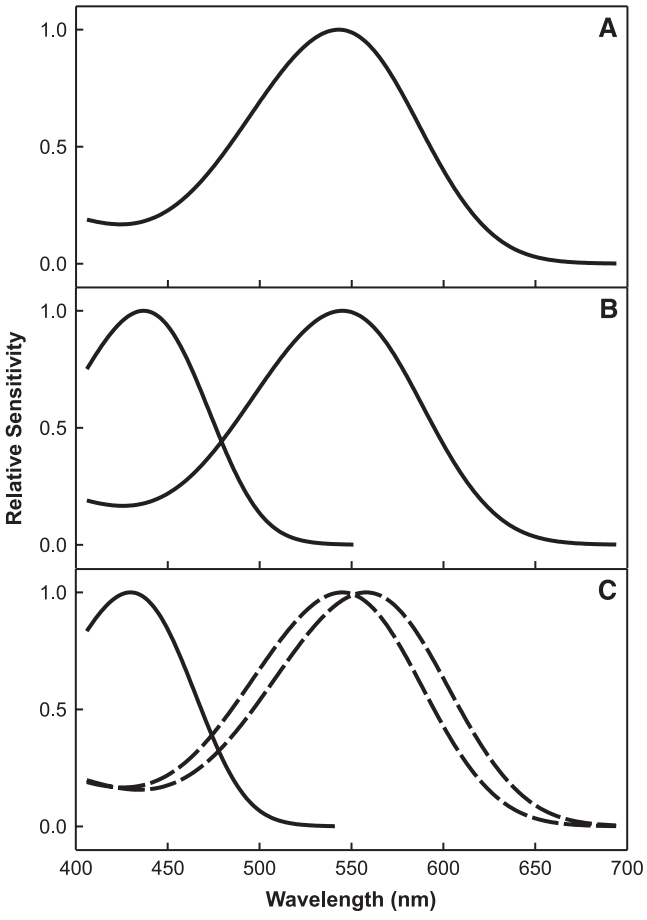

Fig. 5. Spectral sensitivity functions for cone pigments of strepsirrhine primates. The three panels reflect the three patterns described in the text. (**A**) A single type of M/L cone pigment. (**B**) An S-cone pigment and a type of M/L pigment. (**C**) An S-cone pigment and polymorphic versions (dashed lines) of two types of M/L cone pigments. Identities of the species having these variant patterns and the pigment λ_{max} values are given in the text.

primate suggests that the retina should have two functional classes of cone pigment (Perry et al., 2007). Such an arrangement would not be unusual, but a recent brief report suggests that the residues present at tuning sites in the aye-aye SWS opsin gene predict that the short-wavelength cone pigment may have its absorption peak in the UV, not in the visible wavelengths (Hunt, 2006). If true, this would be a unique arrangement for a primate, and that fact alone should make this species an important target for further examination.

Finally, a word about tarsiers. Tarsiers are curious Asian primates whose taxonomic position has been a source of much debate. Although classically lumped together with the others considered in this section (as being, collectively, ''prosimians''), the modern cladistic consensus (Schmitz et al., 2001) is that tarsiers are now properly considered as members of the suborder Haplorhini, apparently more closely related to the anthropoids than to the strepsirrhines (Ross & Martin, 2007). These small nocturnal primates are primitive in many respects, and although they are not included in the primate phylogeny derived for Fig. 2, they have enjoyed an extended period of evolution independent from that of other primate lineages. Recent immunocytochemical labeling experiments identify separate populations of S and M/L cones in the retina of one species of tarsier (Hendrickson et al., 2000), and this suggests that they may have an opsin gene and cone pigment complement similar to that described above for the ring-tailed and brown lemurs.

If the basal condition for primates included an S and a single M/L opsin gene coding for two classes of cone pigment, then at least two types of change must have occurred in the evolution of strepsirrhine lineages: the loss of a population of functional S cones in many species and the acquisition of M/L polymorphism in some others. The timing of the former is uncertain; however, the mutational changes that render the S-cone opsin gene nonfunctional in lorises and bush babies are shared, implying that at least in this case, the loss occurred long ago in an ancestor that was common to the two lineages (Kawamura & Kubotera, 2004). When M/L opsin gene polymorphisms arose in the strepsirrhines is even less clear, but establishing that fact could be important for determining the course of the evolution of primate trichromacy as discussed in the next section.

History of primate opsin gene polymorphism

As noted, sequence comparisons suggest that X-chromosome opsin gene polymorphisms appeared early in the evolution of platyrrhine monkeys. The recent discovery that similar polymorphisms are found among the strepsirrhines raises the question of whether such polymorphisms arose independently in the platyrrhines only after they had diverged from the catarrhines (Fig. 2) or whether polymorphism is a much more ancient feature of primate visual system evolution. The following observations may be relevant to deciding this issue.

(1) On the basis of sequence comparisons of strepsirrhine opsin genes, Tan et al. (2005) suggested that the common ancestor of all primates was polymorphic for the M/L alleles. If that were the case, then opsin gene polymorphism has probably existed throughout primate history, and so its appearance in platyrrhines and some strepsirrhines is simply a contemporary manifestation of a long-standing feature. Since it is not easy to see how M/L pigment polymorphism would promote fitness in nocturnal animals, this claim runs counter to the standard view (above) that ancestral primates were nocturnal. But if, as Tan et al. suggest, polymorphism in fact did arise early in primate evolution, then it was subsequently lost in (a) many lineages that are currently nocturnal, (b) others that are not currently nocturnal but do express only a single M/L cone pigment, and (c) those species that have acquired a second X-chromosome opsin gene.

(2) The same spectral tuning sites are used for all nonhuman primate M/L opsin genes (Table 2), and this coincidence is sometimes taken to suggest that they all may have had a common origin. Against that possibility, it may be that only a handful of amino acid variations can be used to generate M/L spectral absorption differences, and thus their common appearance in different lineages merely reflects not common ancestry but rather convergent evolution.

(3) There is less nucleotide divergence among the allelic forms of the platyrrhine M/L genes then there is between the catarrhine M and L genes (Hunt et al., 2005). That fact would suggest that the platyrrhine polymorphism is more recent than the catarrhine gene duplication and thus that polymorphism did not exist among the early catarrhines. However, sequence homogenization leading to interallelic recombination might also produce this same effect, and thus this difference may not be compelling one way or another.

Taking all these facts together, it seems clear that at present there are sufficient contradictory indicators that make it impossible to decide whether photopigment polymorphism arose early in primate evolution, and then was lost at various junctures, or whether polymorphism emerged only at an early stage in platyrrhine diversification and also, independently, in the more distal branches of the strepsirrhine radiation.

M/L photopigment expression

To provide receptor signals optimal for supporting color vision, each photopigment type needs to be individually expressed in cone receptors. In primates having only a single type of pigment gene on the X-chromosome (e.g., all platyrrhine male monkeys), no problem arises. However, those primates that harbor more than one type of M/L pigment gene require additional specification. One key to assuring individual expression of different pigment types is the dosage compensation mechanism of X-chromosome inactivation, the general control device that has evolved to equalize protein expression in male and female cells in which, during early embryological development, one of the two X-chromosomes is randomly inactivated in female cells, and this inactivation is maintained through all the progenies of each cell. For the heterozygous females of those species that are polymorphic, X-chromosome inactivation neatly solves the problem of selective pigment expression in that one of the two M/L pigments is expressed in roughly half of her cones, while others express the other pigment type (Jacobs & Williams, 2006).

With two different genes on the X-chromosome, catarrhine primates require an additional control to assure the individual expression of cone pigment. That mechanism apparently involves interactions with a region of DNA located upstream to the opsin array, the so-called locus control region (LCR). A series of experiments have established that the LCR pairs with the promoter sequences of either the M or the L gene and, in so doing, determines which gene gets expressed in any given cone cell (Wang et al., 1992; Smallwood et al., 2002). That pairing (illustrated in

Fig. 6A) is believed to reflect a random process and is argued to be the basis from which the position of the M and L cones in the retinal mosaic is, at least across local regions, also effectively random. Beyond the catarrhines, only one other primate, the howler monkey, is known to have two opsin genes on the X-chromosome. The means for expression of M/L cone pigments in these monkeys is less clear, because in these animals rather than there being a single upstream LCR, an LCR is associated with each of the pigment genes (Dulai et al., 1999) (Fig. 6B). Just how this arrangement allows selective receptor expression of the M and L pigments is not known. One possibility, apparently so far unevaluated, is that the downstream LCR has, over time, accumulated changes that render it currently nonfunctional.

X-chromosome opsin gene duplication

Gene duplication has long been recognized as a process central to providing the basis for the evolution of new gene function (Zhang, 2003). X-chromosome opsin gene duplication must have occurred at least twice during primate evolution—at the base of the catarrhine radiation and in an ancestor to present-day howler monkeys. The tandem arrangement of the catarrhine L and M cone opsin genes suggests that they emerged as a result of unequal crossing-over. Logically, this process could have placed two identical cone opsin genes on an X-chromosome, and these two then would have subsequently diverged in structure so as to code for separate M and L opsins. Alternatively, crossing-over might have occurred on the background of a preexisting polymorphic arrangement such that separate M and L genes got co-located on the X-chromosome in a single step. The former process (duplication, then divergence) would be the more typical interpretation (Zhang, 2003), but if polymorphism arose early in primate evolution, as discussed above, then divergence may well have preceded the duplication step. It seems almost certain that the latter did occur in howler monkey evolution since, as noted above, this monkey features duplicate LCRs in the X-chromosome opsin gene array, and such an arrangement suggests that the process of unequal crossing-over must have involved preexisting allelic forms of the M and L opsin genes.

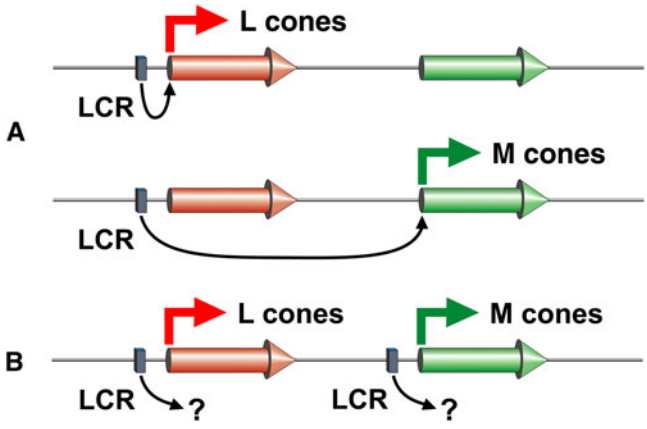

Fig. 6. Illustration of mechanisms proposed to control expression of X-chromosome opsin genes. (**A**) In catarrhine primates, the L and M opsin genes are located in tandem array on the X-chromosome. Experiments suggest that that an upstream LCR pairs randomly (arrows) with the promoters for either the L-cone opsin gene (top) or the M-cone opsin gene (bottom) to assure individual expression of the two pigment types. (**B**) The platyrrhine howler monkey also has L and M opsin genes arranged in a tandem array on the X-chromosome. In this case, there are, as illustrated, two LCRs; it is not known how these two interact with the opsin gene promoters to assure individual expression of pigment into the two cone types (see text discussion).

Evolution of primate color vision

It has long been appreciated that the presence of multiple types of cone pigment is necessary to support a color vision capacity, but it is not sufficient. In addition, the visual system must be organized in a manner that allows for the neural comparison of signals originating in cones containing different pigment types. This section considers some issues linking primate cone pigments to primate color vision.

Inferring the dimensionality of color vision

The first clear statement about the dimensionality of color vision is generally said to be contained in Thomas Young's famous 1802 speculation that the number of fundamental mechanisms underlying human color vision is limited to three (Mollon, 2003). Color mixing experiments conducted later in the 19th century provided an extensive empirical basis for this claim. This fundamental characteristic of human color vision has since that time been described as trichromacy. Directly linking these fundamental mechanisms to the presence of three types of cone photopigments is a more recent accomplishment, as was noted above. Early studies of human color defectives by König and others revealed individuals whose color matches required two not three fundamentals, and these people were termed dichromats (Smith & Pokorny, 2003). Although dimensionality, be it trichromatic or dichromatic, is ultimately defined by color matching measurements, a host of studies of color vision have identified other behavioral indices that covary with dimensionality, for example, results from various types of spectral discriminations, and these are often taken as secondary means to diagnose dimensionality.

Directly measuring color vision in nonhuman subjects is a tedious business usually involving extended periods of training and testing. Because of the compulsive linkage between the number of cone pigment types and the dimensionality of color vision in humans, and because it is relatively more efficient to assess the number of cone types either by direct measurement or by inference from studies of cone opsin genes, it is attractive to automatically assume that the same linkages hold in other species. Enough so that in contemporary reports it has become routine to refer, for example, to evidence for the presence of two cone types as automatically implying dichromatic color vision. At least among the primates, such inferences seem mostly well justified, but there is still room for caution.

For a representative nonhuman catarrhine, the macaque monkey, and for a few species of the polymorphic platyrrhines (e.g., squirrel monkeys, marmosets), there are direct behavioral measurements to compellingly link the presence of two or three types of cone pigment to dichromatic and trichromatic color vision capacities. And the owl monkey, the single anthropoid species known to have only one type of cone pigment, has been shown to be monochromatic (Jacobs et al., 1993). For the strepsirrhines, the story is a little less clear-cut. There have been few direct behavioral studies of color vision in these primates and none that would seem to provide compelling tests of dimensionality that can

be directly correlated with known photopigment complements. Although it seems likely that the dimensionality of their color vision aligns with the photopigment story outlined above, the clear differences in the organization of anthropoid and strepsirrhine retinas suggest that it would be very useful to have this correlation tested directly, ideally in members of one of the species that have a photopigment polymorphism.

A puzzling primate with regard to linking cone complement to color vision is the tarsier. These small animals, variously described as being nocturnal or crepuscular, have enormous eyes with a heavily rod-dominated retina that nevertheless displays a clear fovea. Immunocytochemical labeling experiments identify two classes of cone in the tarsier retina—one UV/S and the other M/L (Hendrickson et al., 2000). There is a significant centroperipheral cone density gradient with an almost reciprocal distribution of the two cone types such that there are very few UV/S cones in the central retina, which then become denser near the retinal periphery, just where the M/L cones are relatively sparsely distributed (Hendrickson et al., 2000). Since extraction of a color signal is classically conceived as depending on a local comparison of inputs from the two cone types, it is not at all clear that this unusual arrangement of the two cone types will support much of a color vision capacity, and thus it may be a bit of a stretch to assume that the tarsier is dichromatic. Although the tarsier is undoubtedly unusual, that case may serve as a clear warning that it is not always a given that one can straightforwardly infer color vision from indicators of the cone pigment complement alone. That caution is particularly apt for cases involving ancestral species for whom development of an opsin gene phylogeny may make it possible to infer the number of cone pigments while at the same time offering no insight into the number and retinal distribution of the cones, nor indeed of any of the other details of retinal construction.

Rod signals and primate color vision

To this point, the discussion has focused exclusively on various issues related to the operation of cone photoreceptors. That is in accord with the traditional linkages made between cone signals and color vision. But there is a considerable span of photic light levels over which rods and cones are jointly operative—in human observers, for instance, this domain (mesopic vision) encompasses a range of as much as four log units of light intensity (Wyszecki & Stiles, 1982). Rod and cone signals share output pathways from the retina, and thus it is probably not surprising that under mesopic test conditions rods have been demonstrated to influence color vision in various ways. These influences are complex, perhaps reflecting the multiple sites available for interaction of rod and cone signals in the retina, and they are not easy to economically summarize, at least partly because they vary depending on numerous viewing parameters such as stimulus size, timing, and retinal location (Buck, 2003). Among the most studied of the rod influences on color vision are the biases they can exert on perceived hue; for instance, in human observers tested at mesopic light levels, rod signals preferentially enhance the percept of blue relative to yellow (Buck et al., 1998). A recently-devised psychophysical technique predicated on the ability to independently control rod and cone stimulation offers the promise of being better able to link appearance measurements such as these to underlying physiology, thus ultimately helping to specify how rod and cone signals interact (e.g. Cao et al., 2005). Finally, it has been apparent for some time that rod signals can also significantly impact the dimensionality of color vision; for example, with relatively large test fields, human dichromats behave trichromatically, that is, they make unique color matches that require the use of three rather than the two primaries that suffice when they are tested at photopic light levels (Smith & Pokorny, 1977).

Although there is thus the clear possibility for rod influences on color vision, there is virtually no indication of how these may manifest themselves in real-world viewing. The opportunity is certainly there given that almost all nonhuman primates are behaviorally active under some conditions of illumination that will be mesopic for their eyes—for instance, during the twilight transitions between day and night and for cases where significant amounts of ambient light are naturally occluded as during periods of heavy overcast or at deeply shaded locations in the subcanopy regions of rain forests. Rod influences on color vision may be even greater for those primates whose visual systems feature certain organizational features, for instance, relatively greater rod representation, the absence of a fovea, reduction in the number of cone types. With regard to this latter item, it is noteworthy that electrophysiological recording studies conducted on both dichromatic and monochromatic platyrrhine monkeys reveal the presence of continued rod contributions to central visual system neurons at very much higher light levels than those typical found in the visual systems of trichromatic primates (Yeh et al., 1995; Silveira et al., 2004). Whether these potential rod influences on color vision are employed adaptively in the conduct of normal primate behavior, and thus may render the mechanisms underlying rod operation subject to selection, or whether they are simply unavoidable by-products of the organization of duplex visual systems is unclear. In sum, although at present not much can yet be said about rod influences on primate color vision in a comparative context, there is a possibility that this topic may comprise an important story still to be written. That point may be particularly apt for the issues covered next.

Nocturnality and primate color vision

A number of strepsirrhine species, as well those from the anthropoid genus *Aotus*, have usually been described in the literature as nocturnal. As noted above, two different cone pigment patterns have been documented in such primates: (1) an SWS1 and an LWS pigment, both seemingly fully functional, and (2) an LWS pigment but no functional SWS1 pigment. The latter arrangement will not permit color vision, but the former might. Although some species (hawk moths and nocturnal geckos) have recently been shown to be capable of making color discriminations at very low light levels, this capacity rests on structural features of their eyes that allow for a sacrifice of temporal and spatial resolution in exchange for a range of spectral resolution that extends down to very low light levels (Kelber & Roth, 2006). Such adaptations are not obviously available in any mammalian eyes, and thus, primate color vision is necessarily limited to light levels that are higher than those present under typical nocturnal conditions. Given this limitation, why would a nocturnal primate maintain a capacity for color vision?

One obvious answer is that the linkage between light level and behavioral activity in animals is seldom as constrained as textbook definitions of nocturnal, diurnal, and crepuscular patterns might suggest. In addition to that, it is well recognized that the activity patterns of various primates are not well accounted by any of these traditional divisions but rather are best described as ''cathemeral,'' a term coined to describe activity that may be distributed throughout

the 24-h cycle with the particulars of the pattern dependent on a wide variety of factors such as ambient temperature, food availability, predation pressure, etc. (Tattersal, 2006). One of the strepsirrhines known to be cathemeral is the brown lemur (*Eulemur fulvus*), an animal that has two functional classes of cone pigment (Jacobs & Deegan, 1993). Clearly, primates such as this will routinely encounter light levels where color vision could be a visual asset.

There are other primate species classically considered to be nocturnal that have two classes of cone pigment and thus at least the potential for dichromatic color vision. The opportunities for exploiting this color capacity seem on the face much reduced in such animals. But they are not absent entirely. Although this will vary from species to species, for humans the luminance of a white paper viewed in full moonlight is about double the cone threshold (although perhaps still not high enough to reach the threshold for color vision), and there is the suggestion that under such conditions, bright saturated colors may be visible (Makous, 2004). Thus, there is at least the marginal possibility that some ''nocturnal'' conditions may support limited color vision. Probably more relevant, it is well documented that even the most devoutly nocturnal primates will occasionally awaken and become active during daylight hours; for example, in response to the presence of predators, to respond to weather contingencies, or simply to initiate searches for sustenance in times of food shortage (Bearder et al., 2006). Such circumstances, even if relatively infrequent, may be sufficient to support the maintenance of a minimal color vision capacity.

The above examples suggest that there are no facile linkages to be made between the presence of color vision and photic activity cycles. That conclusion is made even stronger by observations on animals that have the second of the two arrangements, that is, only a single type of cone pigment and a consequent absence of color vision. One such example is the owl monkey *Aotus azarae*. This species, like others of the same genus, lacks a functional SWS1-based pigment (Levenson et al., 2007). Surprisingly, although most species of *Aotus* are nocturnal, *A. azarae* is cathemeral, often seen to be active during daylight hours as well as during the night (Fernandez-Duque, 2003). The daylight light levels encountered by this monkey are such that its vision will be mostly based on photon capture by a single cone photopigment. Interestingly, the daytime diet of *A. azarae* includes, among other things, brightly colored tree flowers (Gimenez & Fernandez-Duque, 2003). Observers of such foraging behavior might be tempted to conclude that the flower harvest is guided by color cues, perhaps then proceeding to predict what array of cone pigments might best subserve this discrimination. Of course, this monkey has no capacity to exploit such spectral cues and thus this case underlines the difficulty of inferring a capacity for color vision solely from observations of normal behavior. It additionally makes clear that a complete lack of color vision need not present an insurmountable barrier to primate success under daylight illumination.

Given the prospect that nocturnal primates are sometimes in environmental circumstances that support the use of color vision, perhaps the real question is not why some nocturnal primates have maintained color vision but, rather, why some lineages have seemingly abandoned that possibility through the accumulation of mutational change in their SWS1 opsin genes? Although the loss of one class of functional cones does not need to have been adaptive, its spread to include all members of the species allows the possibility that it may have been. But if so, it is not obvious how this loss might enhance primate fitness and so, at present, this gene-induced abandonment of a potential dimension of color vision in select primate lineages remains very much a mystery.

Retinal pathways supporting primate color vision

The extraction of color information is initiated in retinal circuits organized to contrast photon absorption patterns in cones containing different types of pigment. In mammals, two types of such circuits have been identified (for reviews, see Martin, 1998; Masland, 2001; Lee, 2004; Wässle, 2004). One incorporates a dedicated class of bipolar cells that selectively receive inputs from S cones. Signals from these bipolar cells are fed to small bistratified ganglion cells, which also receive inputs from a group of bipolar cells that contact M/L cones. Signal inputs from these two sources are combined in an antagonistic fashion to form the basis for the blue/yellow opponent pathway. Although the number of species in which this pathway has been definitively demonstrated thus far is limited (e.g., catarrhine and platyrrhine primates: Dacey & Lee, 1994; Silveira et al., 1999; some rodents: Haverkamp et al., 2005; Li & DeVries, 2006; and rabbit: MacNeil & Gaul, 2008), it seems reasonable to suppose that this arrangement is characteristic of the eutherian retina, implying that it has been maintained throughout the course of most of mammalian evolution. Since early mammals are assumed to have been nocturnal, the apparent antiquity of this pathway adds weight to the idea explored above that even for animals that are principally nocturnal, some color capacity provides an adaptive advantage (although, it should be said, there may be reasons beyond support for color vision for maintaining two classes of cone). For contemporary mammals that maintain separate populations of S and M/L cones, these ganglion cells provide the initial neural substrate from which a single dimension of color vision can emerge. Thus far, we do not seem to know the fate of this pathway in those primates, such as the *Aotus* monkey, that have lost their S cones to opsin mutation.

The second retinal circuit for extracting color information is unique to primates. It originates as M or L cone inputs to midget bipolar cells, and these in turn synapse on midget ganglion cells (Martin, 1998). Classically, this arrangement was conceived to provide signal input from a single type of foveal cone (M or L) to a single ganglion cell, which was then combined antagonistically with signals that originate from activation of M and L cones in neighboring regions to form the red/green opponent pathway. There is now evidence that signals from more than one foveal cone may be collected by an single ganglion cell (McMahon et al., 2000), and it is well established that the receptive field centers of midget ganglion cells in the retinal periphery encompass an area sufficient to contain inputs from many cones (Dacey, 1993; Goodchild et al., 1996). Currently, there are also continuing questions about the purity of the cone signal to the surround regions of the red/green opponent cells and concern as to how these center/surround arrangements may be altered for ganglion cells located in the retinal periphery. Whatever the eventual resolution of all these issues, it is clear that it is from signals of cells of this type that a second dimension of color vision is extracted by the primate central visual system (Solomon & Lennie, 2007).

The addition of a second class of M/L cone and the presence of midget cell circuitry permit the emergence of primate trichromacy. What is the evolutionary linkage between these two critical

elements? Since the presence of a second, spectrally discrete M/L pigment will automatically expand the spectral window through which an animal samples the photic environment, one possibility is that pigment addition *per se* could yield visual advantages and, if so, the circuitry to compare outputs from the two pigment types might have been evolved subsequent to the addition of a new pigment type. A more popular idea, however, is that the pathways to channel signals from single cones to ganglion cells first emerged as an adaptation to support higher spatial acuity. With this circuitry in place, the subsequent addition of a new M/L cone type would then automatically allow for an opponent comparison of signals from M and L cones, thus supporting an immediate step to trichromacy (e.g., Wässle & Boycott, 1991). Perhaps weighing against this possibility, Lee (2004) has pointed out that the spatial vision that can be derived from single-cone inputs to midget ganglion cells would exceed the resolution capacity of primate optical systems. In his view, this could make it unlikely that the midget cell system, at least as it is currently configured in primate retinas, would have evolved solely as an adaptation to support high spatial acuity.

Comparative studies shed some light on these issues, but they do not resolve them. Comparisons of retinal ganglion cell anatomy and physiology in catarrhine and platyrrhine primates show that the retinal circuitry in representative species from the two groups is very similar and that, further, except for differences in spectral sensitivity, dichromatic and trichromatic conspecific platyrrhines also have much the same midget cell organization (Kremers & Lee, 1998; Silveira et al., 2004). These observations imply that the postreceptoral arrangements sufficient to support trichromatic color vision were already in place in the anthropoid ancestral to these two lineages. Perhaps even more surprising, anatomical studies further suggest that a retinal midget cell system is also present in the strepsirrhine bush baby (Yamada et al., 1998). The apparent ubiquity of this system across contemporary primates would imply that either this retinal organization arose long before trichromacy first emerged, perhaps to support some other visual need such as enhanced acuity, or, as has been suggested (Tan et al., 2005), trichromacy emerged earlier in primates than is usually believed.

Why among mammals are the primates uniquely trichromatic?

Either as a routine capacity or as a polymorphic alternative, trichromacy has been detected in animals from each of the three major primate groupings. As far as is known, however, no other eutherian mammals are trichromatic (Jacobs, 1993). What accounts for this disparity? It cannot be just a lack of sufficient cone population for, although some mammals indeed have sparse cone representation (e.g., comprising no more than ~1% of all photoreceptors), a number of mammalian lineages feature retinas with cone:rod ratios that exceed those of any primate—often very greatly, as, for example, in the ground squirrel (Kryger et al., 1998) or the tree shrew (Müller & Peichl, 1989). Neither can it be traced entirely to photic lifestyle since, although there are to be sure plenty of nocturnal mammals, there are numerous mammals that have been variously characterized as diurnal, crepuscular, or arrhythmic (Macdonald, 2001), nor does there seem any reason to believe that the likelihood of mutational changes in opsin gene tuning sites that would lead to the emergence of new cone pigments should have been significantly greater in the primates than in other mammals. These possibilities aside, the explanation most frequently offered focuses on the absence of retinal midget cell systems in nonprimate retinas. Boycott and Wässle (1999) summarized the idea that a pigment gene mutation leading to separate populations of L and M cones may not, by itself, be sufficient to produce new color vision, by noting that "The split might result in equal numbers of L- and M-cones randomly distributed across the retina. In mammals, other than primates, bipolar cells pool the signals of several neighboring cones and, in turn, ganglion cells pool the signals from several converging bipolar cells. In such a highly convergent system the chromatic information introduced in the cone mosaic by the L/M mutation would be lost within the retina and thus never reach the brain." If this idea is right, then even if new M/L pigments should appear in nonprimate mammals, they might not evolve.

In recent years, attempts have been made to study this issue directly by using genetic engineering to introduce a second M/L photopigment into the retinas of mice, animals that, like most other mammals, normally express only one such pigment (in this case, λ_{max} = 510 nm). In a first attempt, mice were generated that were transgenic for a human L-cone opsin gene (linked to the production of a pigment with λ_{max} of 556 nm). In these animals, the transgene was incorporated autosomally with the result that ~80% of the cones coexpressed human L- and the native mouse M-cone pigment (Shaaban et al., 1998). Spectral sensitivity curves for the full complement of cone pigments in these mice are shown in Fig. 7A. Electroretinogram (ERG) recordings showed that the human cone pigment worked efficiently in mouse cones, yielding changes in outer retinal signals consistent with the operational

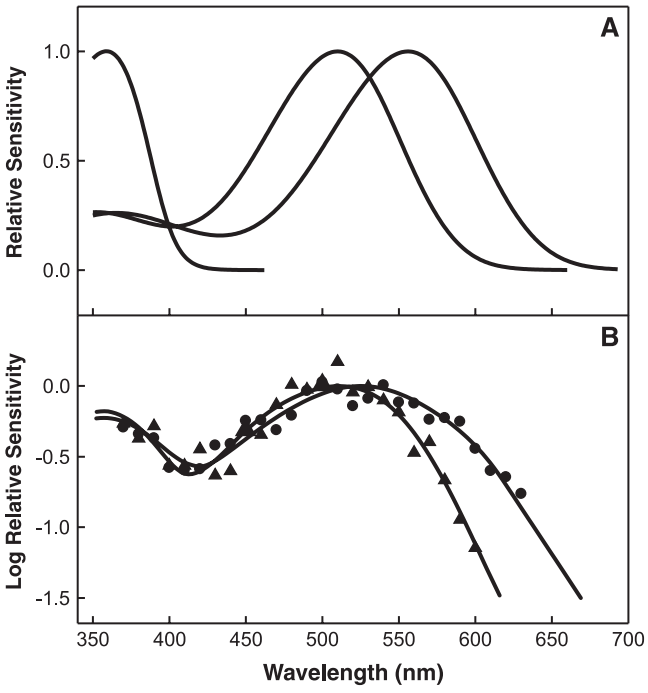

Fig. 7. Results from experiments to evaluate the functional consequences of adding a cone pigment to their normal pigment complement of the mouse. (**A**) Spectral sensitivity curves for the three types of cone pigments found in two types of bioengineered mice. The native pigments in the mouse have λ_{max} values of 360 and 510 nm, while that for the added pigment is 556 nm. (**B**) Behavioral increment threshold functions obtained from wild-type mice (triangles) and transgenic mice (circles). In addition to the two cone pigments found in wild-type mice, most cones of the latter animal also expressed a human L-cone photopigment. The continuous lines are best-fit linear summations of the absorption curves for cone pigments found in each type of mouse. Results derived from Jacobs et al. (1999).

presence of an L-pigment (Jacobs et al., 1999). Behavioral tests conducted to determine if the new pigment impacted the visual capabilities of these mice showed that they do, as illustrated in Fig. 7B, which shows increment threshold spectral sensitivity equivalently measured in wild-type and transgenic mice. These transgenic mice acquired significantly enhanced sensitivity to long-wavelength lights, much as would be predicted by the added presence of a cone pigment with a peak of 556 nm. A direct test to illustrate the magnitude of this change showed that these transgenic mice could detect light at least 30 nm further into the long wavelengths than wild-type mice. Finally, although extensive tests for color vision were run, the transgenic mice consistently failed to evidence any such capacity. Because the novel pigment was coexpressed in cones containing the native pigments, the absence of color vision is probably not surprising. Although no new color vision emerged from the mere addition of a second M/L pigment in this manner, clear alterations in visual sensitivity were produced. As I have noted, changes like this might, under the right circumstances, provide an adaptive advantage.

To avoid the problem of pigment coexpression, two more recent investigations targeted the human L-opsin gene to the mouse X-chromosome in knock-in mice (Smallwood et al., 2003; Onishi et al., 2005). The gene/pigment arrangement in such mice was specifically designed to mimic that of the polymorphic New World monkeys in that three separate M/L pigment phenotypes are generated by this procedure—mice having either the native M or human L pigment (all males and homozygous females) or animals expressing both M and L pigments in separate cone populations (heterozygous females). As in the case of the transgenic mice, ERG recordings showed that the human L-pigment efficiently transduces light in mouse photoreceptors and that spectral sensitivity based on outer retinal signals was consistent with predictions from the new opsin gene complements. Subsequently, extensive behavioral tests were conducted to establish if vision was also altered in these mice (Jacobs et al., 2007). As with the transgenic mice, knock-in mice expressing the human L cone pigment showed predictable increases in long-wavelength sensitivity. Unlike the transgenic mice, however, the behavior of several of the heterozygous knock-in mice indicated the presence of a color vision capacity absent in control mice. Wavelength discrimination functions derived for three such animals (Fig. 8) give one illustration of the nature of the new color vision.

The results illustrated in Fig. 8, along with additional color matching tests, show quite clearly that color vision, as that capacity is formally defined, can be produced by the addition of a novel cone pigment to the normal cone complement of a mammal. Three implications can be drawn from this demonstration. First, these results reinforce the view that visual systems have a remarkable plasticity in that a simple alteration in first-stage receptor molecules can, all by themselves, trigger the emergence of a complex new sensory capacity. Second, the presence of a retinal midget cell system like that characteristic of primate retinas must not be an inescapable prerequisite for adding a new dimension of color vision. Third, as was suggested early in this line of research (Mollon et al., 1984), this result implies that the addition of a novel M/L cone pigment through opsin gene alterations probably allowed our primate ancestors to immediately gain new color vision.

These results also come with several caveats. First, despite the fact that all the heterozygous mice had populations of both M and L pigments in their retinas, only some animals seemed to acquire color vision (Jacobs et al., 2007). Whether this partial pattern of success in the color vision tests has a mundane explanation (e.g., some inadequacies in the training regimens) or whether it reflects something more basic is not known. Second, even those animals that did achieve novel color vision required thousands of training trials before consistent color discriminations began to appear. What this may mean is also unclear; one possible interpretation is that the color cues newly available to these knock-in mice are not very salient, and thus, it is difficult to encourage the animal to make use of them. Third, these experiments do not explicitly address the question of how the mouse visual system is able to extract this new color signals. Many mouse ganglion cells have spatially antagonistic center/surround receptive fields (Sagdullaev & McCall, 2005), so one possibility is that in these knock-in mice there were sufficient differences in the L and M cone representation into these two regions to yield spectrally opponent signals. Alternatively, some upstream comparison of the relative M and L signal weightings across ganglion cell populations might also allow for the extraction of a color signal. Well-directed recording experiments could reveal just how these heterozygous mice extract and exploit the new color signal.

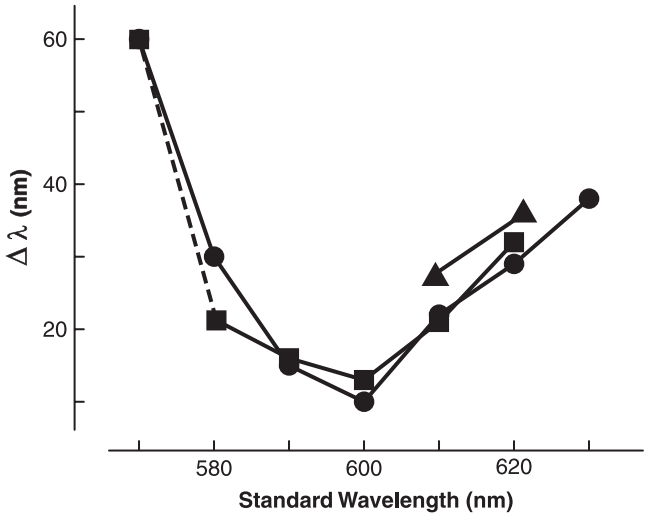

Fig. 8. Wavelength discrimination functions for heterozygous knock-in mice whose retinas contain all three of the photopigments whose spectra are plotted in Fig. 7A. Each point plots the difference in wavelength (in nanometers) required for successful discrimination at each of the seven spectral locations. These results illustrate the clear presence of novel color vision in these mice that is traceable to the presence of the added cone photopigment. The separate symbols represent results from different animals (data from Jacobs et al., 2007).

Given that these knock-in mice were able to add new color vision by exploiting the artificially induced presence of a new type of cone pigment, one wonders why a similar change did not happen in some mammalian lineages during the course of their evolution. To start with, we cannot be absolutely confident that it has not occurred, being present somewhere among that significant cohort of mammals whose cone photopigment and color vision status remain to this day uninvestigated. And there is also the possibility that even if an opsin gene change had yielded a new cone pigment and the nervous system had been capable of generating new color vision, that capacity may not have altered species fitness sufficiently for it to be maintained over subsequent generations. Another possibility is that in the absence of a midget cell system, cone and ganglion cell density may critically influence the prospects for extracting a spectrally opponent signal.

For instance, mammals having very high cone densities may be unlikely candidates for acquiring new color vision in this way since, much in the fashion described by Boycott and Wässle (quoted above), the mixed convergence of signals from the two pigment types would minimize the possibility of allowing strong ganglion cell spectral opponency. Somewhat paradoxically, a sparser cone representation may provide a more favorable substrate for generating a novel color signal in these retinas. In the knock-in mouse, for example, X-chromosome inactivation resulted in the appearance of patch-like clusters of M and L cones containing the same pigment type (Smallwood et al., 2003; Onishi et al., 2005). The patch sizes so generated are not much smaller than the graininess of ganglion cell receptive fields, and this approximate matching may have been critical for permitting useful spectral comparisons between the two cone types.

Although we do not yet know in detail what factors may eventually be required to explain why trichromatic color vision emerged in primates but not in other mammals, it seems indisputable that the primate midget cell provides a remarkably efficient neural substrate for setting up the initial comparisons between different cone types that are required to support M/L color vision. Whether these pathways initially evolved as an adaptation to support higher spatial acuity or whether they evolved in conjunction with very early primate trichromacy, the primate midget cell system has fostered flexibility for color vision change in primates that is not available in other mammalian visual systems.

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