Evolution and selection of trichromatic vision in primates

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Trichromatic colour vision is of considerable importance to primates but is absent in other eutherian mammals. Primate colour vision is traditionally believed to have evolved for finding food in the forest. Recent work has tested the ecological importance of trichromacy to primates, both by measuring the spectral and chemical properties of food eaten in the wild, and by testing the relative foraging abilities of dichromatic and trichromatic primates. Molecular studies have revealed the genetic mechanisms of the evolution of trichromacy, and are providing new insight into visual pigment gene expression and colour vision defects. By drawing together work from these different fields, we can gain a better understanding of how natural selection has shaped the evolution of trichromatic colour vision in primates and also about mechanisms of gene duplication, heterozygote advantage and balancing selection.

Among eutherian mammals, primate vision is unique. Across primate taxa, colour vision is remarkably diverse and studies of how and why such vision has evolved span many fields in ecology and evolution. Although vision is clearly of interest to those studying primate behaviour and forest ecology, the underlying genetics of colour vision provide important insights into polymorphism and population genetics and also into the evolution of gene function and regulation.

Our knowledge of the genetic basis for colour vision now enables us to understand how it evolved, but much work still focuses on why colour vision evolved. Such theories are often controversial, and can be difficult to test. One long-standing hypothesis is that enhanced colour vision in primates evolved to detect ripe fruit on a dappled background of leaves [1,2]. In the past few years however, this has been challenged by the idea that foraging for young leaves, which are often red in colour, explains such adaptive variation [3,4]. A recent finding indicates that primate colour vision might have evolved much earlier than was previously thought, and provides some evidence that an ancestral primate was possibly diurnal rather than nocturnal [5]. However, paleontologists and taxonomists tend to disagree [6]. Interestingly, in some primate taxa, the variety of colours that can be discriminated differs, even among individuals of the same species and sex. This polymorphism enables a direct comparison of individual primates with a variety of colour vision phenotypes to be made, and links differences in a single gene to changes in behaviour. Emerging experiments can now test models of natural selection that were first proposed some 20 years ago [7]. These studies follow the behaviour of individual animals whose genotype and phenotype are known, and provide an exciting way forward for understanding the mechanisms of selection.

Seeing colours

Vertebrate colour vision requires both the presence of PHOTORECEPTOR (see Glossary) cells, called CONE CELLS, in

Glossary

Allelic trichromacy: trichromatic vision achieved through the presence of multiple alleles at the single X-linked opsin locus. Only females can be trichromatic under this system.
Anthropoid: a member of the Anthropoidea, which contains the Platyrrhini (New World monkeys) and Catarrhini (Old World monkeys and apes).
Balancing selection: a process of natural selection that acts to maintain different alleles in a **population**.
Catarrhine: a member of the infra-order Catarrhini, the Old World monkeys and apes.
Cone cell: one of the two main classes of photoreceptor found in the vertebrate eye (rods are the other class).
Dichromatic: two primaries are needed to match nearly any light (in practice it is not possible to find real primaries that will match any light). Requires two spectral receptor types and appropriate neural mechanisms.
Gene conversion: a process of directed change at meiosis in which one allele directs the conversion of a homologous allele to its own form.
Haplorhine: a member of the Haplorhini, which combines the tarsiers and the Anthropoidea.
Heterosis: heterozygotes in a population having higher fitness than homozygotes.
Lyonization: random inactivation of one X chromosome in mammalian cells containing two X chromosomes.
Opsin: photopigment protein of the rhodopsin family.
Photopigment: pigment capable of stimulation by light.
Photoreceptor: cell that reacts to a light stimulus.
Platyrrhine: a member of the infra-order Platyrrhini, the New World monkeys.
Prosimian: a member of the Prosimii, a traditional grouping comprising the tarsiers and strepsirhines (lemurs and lorises). The Haplorhini provides an alternative, phylogenetically correct, grouping of the tarsiers with the anthropoids.
Routine trichromacy: trichromatic vision achieved with separate loci for each of three opsins, leading to trichromacy in all individuals.
Spectral sensitivity: the (relative) effectiveness of a photon of a given wavelength in producing a neural or behavioural response.
Strepsirhine: a member of the Strepsirhini, which contains the lemurs and lorises.
Trichromatic: three primaries are needed to match nearly any light (in practice it is not possible to find real primaries that will match any light). Requires three spectral receptor types and appropriate neural mechanisms.

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the retina of the eye, and neural mechanisms for comparing the responses of these cone cells to generate a colour signal (Box 1). Cones contain visual pigments, known as PHOTOPIGMENTS, which differ in the wavelength of light to which they are sensitive [8]. This is known as their 'SPECTRAL SENSITIVITY'. An increased number of photopigments, with different sensitivities, enables a greater number of comparisons to be made and, hence, increases the potential for colour vision. The behavioural ability of an animal to discriminate colours cannot be inferred directly from knowledge of these photoreceptor sensitivities [8], but, in behavioural tests, primates with two types of cone have colour vision resembling red–green colour-deficient humans (DICHROMATIC), and those with three types have colour vision resembling that of humans with normal colour vision (TRICHROMATIC) [8,9].

Studies of modern representatives of basal vertebrate groups (e.g. the sturgeons, Chondrostei) suggest that the common ancestor of teleost fish and amniotes had four types of photopigment, as do contemporary birds and many reptiles [10]. However, colour vision is relatively poor at low light intensities, and so it is presumed that, during the stages of evolution when early mammals were nocturnal, two of the four ancestral types of photopigment were lost. Most eutherian mammals therefore have two types of photopigment, conventionally known as short-wavelength sensitive (S) and long-wavelength sensitive (L), and are dichromatic. Primates alone have evolved trichromatic vision, and have done so in two different ways.

The molecular basis of primate colour vision

In all primates, the S photopigment is encoded by an autosomal gene, whereas the L photopigment is encoded by the X chromosome. In Old World ANTHROPOID primates (CATARRHINES) and the New World howler monkeys *Alouatta* spp., trichromacy arose following a duplication of the L photopigment gene on the X chromosome. This resulted in divergent genes encoding a separate L photopigment and an additional photopigment of shorter middle-wavelength (M) [11–13]. Their vision is therefore based on three photopigments (S, L and M) and is referred to as 'ROUTINE' TRICHROMACY. However, in most New World primates (PLATYRRHINES), there is a single

Box 1. Photoreceptor spectral sensitivities and primate colour vision

Generating a colour signal in the brain requires cells that are sensitive to different wavelengths of light and a mechanism for extracting and comparing the resulting output from these cells.

Spectral sensitivity

The spectral sensitivity of a photoreceptor cell is defined as the (relative) likelihood of a photon of wavelength λ being transduced to produce a neural signal. In primates, a given photoreceptor expresses one type of photopigment molecule. This photopigment comprises an opsin protein and a prosthetic group, retinal. Retinal absorbs light and, within mammals, variations in spectral tuning of the photopigment depend upon the opsin protein. The spectral sensitivity of a photopigment is accurately specified by the wavelength of the sensitivity maximum, λ_{max}; therefore, pigments can be defined by λ_{max}. Thus a 565nm opsin produces a visual pigment with λ_{max} 565nm.

Nomenclature of cone photopigments and photoreceptors can be confusing. Following convention, for dichromat eyes, the two types of cone receptor are called long- (L) and short-wavelength (S) sensitive, whereas trichromat eyes have an additional middle-wavelength (M) sensitive receptor. L, M and S receptors are sometimes called 'red', 'green' and 'blue' respectively, although this is somewhat misleading because the receptors themselves are colourblind, and their sensitivity maxima are not at wavelengths ordinarily recognized by these colour names. Figure Ia illustrates the normalized spectral sensitivities of the three photoreceptors in the catarrhines and howler monkeys, which have separate M and L opsins. The spectrum indicates the approximate colour as a function of wavelength.

Colour vision

An animal with colour vision can discriminate lights of differing spectral composition, regardless of their intensity (i.e. 'brightness'). This requires both photoreceptors of differing spectral sensitivity and opponency mechanisms for comparing their outputs [62]. Spectral information encoded by an eye with *n* spectral types of photoreceptor can be represented by one 'achromatic' (or brightness) mechanism, which sums receptor outputs, and $n - 1$ 'chromatic' mechanisms. Figure Ib illustrates these chromatic interactions in a trichromatic primate [63]. The phylogenetically older 'blue–yellow' mechanism (grey lines) compares S cone signals with the combined signals of both the L and M cones. Trichromats also have a 'red–green' mechanism (black lines), which compares only L with M cone signals. The amplitude

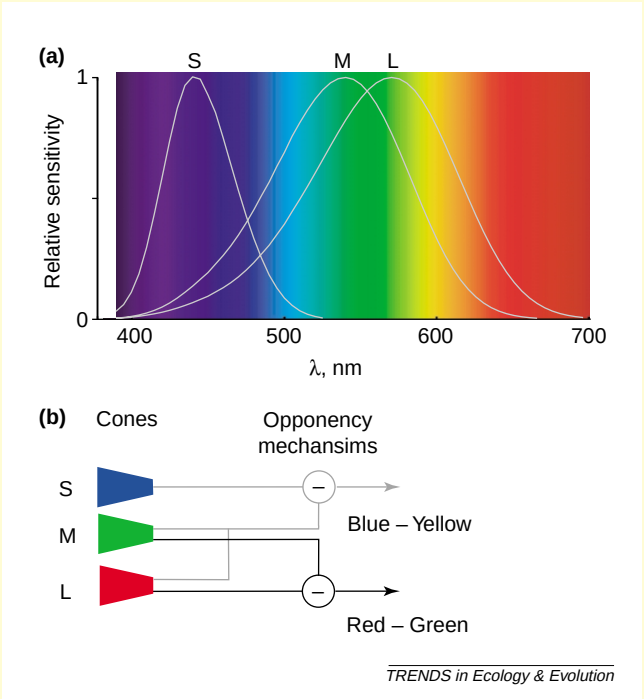

Fig. I.

of these chromatic signals is strongly dependent on the separation of λ_{max} of the inputs, and on the relative numbers of cones. A small separation will give weak signals, and this can limit the number of photopigments that are useful.

Sometimes, eyes are referred to as being di- or trichromatic according to the number of spectral types of cone photoreceptors present. This is not strictly accurate, as the 'chromacy' of vision should refer to the number of primary light stimuli ('primaries') that are required to match nearly any light and therefore depends upon neural mechanisms (in practice it is not possible to find real primaries that will match any light) [62].

photopigment gene on the X chromosome, but this gene is polymorphic and encodes different M to L photopigments. Because the gene is X-linked, heterozygous females are trichromatic with homozygous females and males being dichromats (and therefore are red–green colour blind) [2,14]. This type of trichromacy is referred to as 'ALLELIC' TRICHROMACY. Allelic trichromacy also occurs outside of the anthropoids, as was discovered very recently with the use of DNA sequence analysis. Two different species of STREPSIRHINE have more than one M or L photopigment allele (Table 1) [5], but, although this gives them the genetic potential, trichromacy has yet to be demonstrated behaviourally.

Photopigments consist of an OPSIN protein and retinal (Box 1). Cone opsin proteins generally comprise 364 amino acids and just a few amino acid changes underlie the variation in spectral sensitivity of the pigments, or their 'spectral tuning'. For example, in the catarrhines, substitution with hydroxyl-bearing amino acids at sites 180, 277 and 285 shifts the spectral sensitivity to longer wavelengths by ~5, 8 and 15 nm respectively [15,16].

The M and L genes of the routinely trichromatic howler monkeys use the same amino acids for spectral tuning as do the catarrhines [16], and have photopigments with virtually the same spectral sensitivity (Box 1) [17]. In platyrrhines with allelic trichromacy, differences in spectral tuning between the various X-chromosome alleles are also attributable to mostly the same important amino acid sites as in the routine trichromats [18]. In the strepsirhines, spectral tuning appears to involve one amino acid at site 285 [19]. Therefore, spectral tuning of photopigments among the different primate lineages is remarkably conservative.

Our knowledge of the distribution of photopigment types among different primate groups has greatly increased over the past 20 years (Table 1). This has added considerably to our understanding of how trichromacy has evolved, and has revealed some surprises. The new discovery of the potential for trichromacy in primitive

Table 1. Distribution of types of colour vision and approximate λ_{max} of photopigments, where known, in different primate genera

Family	Genus	Short- wavelength photopigments	Middle- and long-wavelength photo pigments	Vision[a]	Refs
Lemuridae	*Eulemur*	437[b]	543[c]; 545[b]	D	[5,41]
	Lemur	437[b]	543[c]; 545[b]	D	[5,41]
	Hapalemur		558[c]	D	[5]
	Varecia		543, 558[c]	P	[5]
Indridae	*Propithecus*	430[b]	543, 558[c]; 545, 558[b]	P	[5,19]
Cheirogaleidae	*Microcebus*		558[c]	D	[5]
	Cheirogaleus		543[c]	D[g]	[5]
Daubentoniidae	*Daubentonia*		543[c]	D	[5]
Galagonidae	*Galago*	N/A[f]	543[c]	M	[5,42,43]
	Otolemur	N/A[f]	543[c]; 544–545[b]	M	[5,42–44]
Loridae	*Loris*		543[c]	D	[5]
	Nycticebus		543[c]	D	[5]
	Perodicticus		543[c]	D	[5]
Tarsiidae	*Tarsius*		543 or 558[c]	D	[5]
Callitrichidae	*Callithrix*	425[d]	543, 556, 563[c,d]; 539, 553, 561[e]	P	[10,14,45,46]
	Cebuella		556, 563[c]	P	[10,14,23]
	Callimico		543, 563[c]	P	[23]
	Leontopithecus		543, 556, 563[c]	P	[10,14,23]
	Saguinus	433–436[b]	543, 556, 563[c]; 545, 557, 562[b]	P	[47,48]
Cebidae	*Aotus*	N/A[f]	543–545[b]	M	[42,49]
	Saimiri	433[d]	535, 550, 562[c,d]; 543, 550, 561[b]	P	[10,48]
	Cebus	433[d]	550, 562[b]; 535, 550, 562[c,d]	P	[10,18,50]
	Alouatta		530, 562[b]	T	[17]
	Lagothrix	437[b]	548, 563[b]	P	[51]
	Ateles	432[b]	550, 562[b]	P	[51]
	Callicebus		530, 536, 542, 549, 561[b]	P	[50,52]
Cercopithecidae	*Macaca*	430[b]	535, 562[b]	T	[53]
	Cercopithecus	430[b]	535, 562[b]	T	[53,54]
	Miopithecus	430[d]	535, 562[c]	T	[54,55]
	Colobus	430[b]	535, 562[b]	T	[53]
	Presbytis	430[b]	535, 562[b]	T	[53]
Hylobatidae	*Hylobates*		530, 563[c]	T	[56]
Hominidae	*Pongo*		535, 565[c]	T	[57]
	Pan	430[b]	535, 562[b,c]	T	[55,57,58]
	Homo	420[d]	535, 562[c]	T	[59,60]
	Gorilla	430	535, 562[c]	T	[55,57]

[a]Abbreviations: M, monochromacy; D, dichromacy; P, allelic trichromacy (within-species polymorphism); T, routine trichromacy. Blank boxes indicate that no data are available.
[b]Results obtained from DNA sequencing.
[c]Results obtained from electroretinogram.
[d]Results obtained from microspectrophotometry.
[e]Results obtained from *in vitro* expression.
[f]No photopigment is thought to exist because of deleterious mutations in the DNA sequence [44].
[g]*Cheirogaleus major* has one M to L opsin allele and not two as previously reported [5] (Ying Tan, pers. commun.).

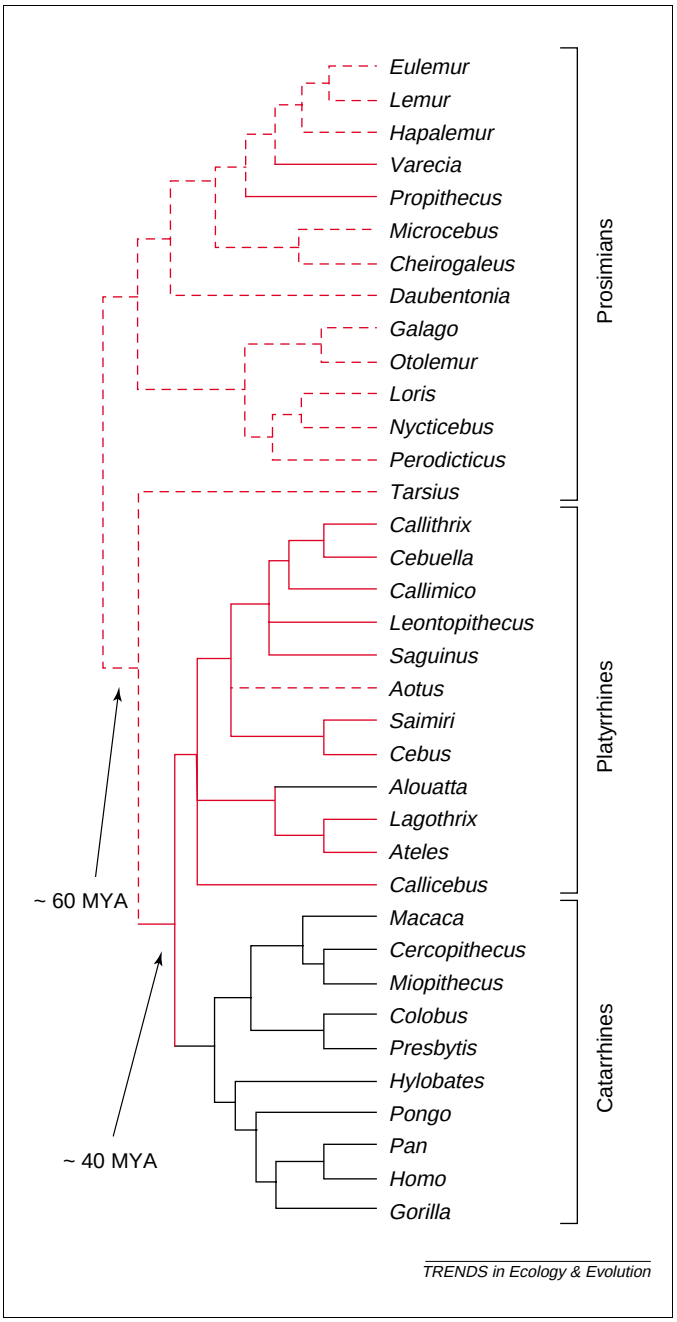

Fig. 1. Colour vision in primates. Primate phylogeny (a consensus of recently published primate phylogenies, e.g. [61]) showing just one of the possible evolutionary reconstructions of colour vision. Dashed line, mono and dichromatic lineages; red line, polymorphic lineages with allelic trichromacy; solid black line, routinely trichromatic lineages.

primate groups has major implications for the reconstruction of earlier events leading to its evolution in primates.

Evolution of colour vision in primates

When did routine trichromacy evolve?

Did routine trichromacy arise early in primate evolution followed by its loss in some primate taxa? All catarrhines and New World howler monkeys have a gene duplication that enables them to have trichromatic vision [16]. However, evidence from DNA sequences of the promoter regions of the M and L genes [12], the 5′ flanking regions and locus control regions [13], indicate that the opsin gene duplication in howlers is independent and more recent than that in Old World primates. Hence, routine trichromacy has evolved independently in the Old and New World monkeys, and is unlikely to have been present before either the catarrhine–platyrrhine primate divergence or the HAPLORHINE–strepsirhine divergence (Fig. 1).

Duplicate and diverge, or diverge and duplicate?

Models of gene duplication generally assume that functional divergence of gene copies follows duplication [20]. Alternatively, functional divergence of alleles might precede the duplication event, with these different alleles becoming 'fixed' as different loci by duplication. This is precisely what appears to have happened in the evolution of routine trichromacy in howler monkeys. The similarity of howler monkey M and L genes to opsin alleles in other New World species with allelic trichromacy, indicates that these genes are derived from different opsin alleles that were present before the duplication event [16]. The sequence of events was presumably as follows: an unequal crossover event during DNA replication in a heterozygous female led to male and female offspring containing separate M and L opsin genes on the X chromosome. These M and L genes were somehow differentially expressed in different cone cells (Box 2), leading to trichromacy. This would have provided these individuals with an immediate selective advantage, and enabled the spread of the X chromosome containing the duplication through the entire population, giving howlers routine trichromacy. Did the polymorphism also exist before the gene duplication in the catarrhines? If divergence of M and L opsins occurred after duplication of a single gene, then the duplicated gene must have persisted in the population for long enough in the absence of selection for divergence to occur. The lack of variable sites between the L and M genes of Old World primates has led to the conclusion that they diverged after the duplication of a single allele [16]. However, GENE CONVERSION is known to occur frequently among X chromosome opsin genes, and this will act to homogenize progressively sequence variation [21,22]. Consequently, it is possible that the duplicated Old World gene originated from different allelic forms (similar to the howler monkey), but, during the longer period of evolutionary time that has elapsed, the neutral differences between the two genes have been lost because of gene conversion or recombination events.

Did polymorphism evolve more than once?

Until recently, polymorphism was thought to be restricted to Neotropical anthropoids, but allelic variation has now been found in some strepsirhine species [5,19]. Hence, the polymorphism might have been much more widespread in the past than previously thought. In addition, the M and L genes are apparently distributed among species in those families with a single allele (e.g. the Philippine tarsier *Tarsius syrichta* has an L gene whereas the western tarsier *T. bancanus* has an M gene). This might imply that a PROSIMIAN ancestor had both M and L genes and was potentially trichromatic [5]. Arguing against this conclusion, Heesy and Ross [6] reconstruct the activity patterns of fossil primates by inferring eye size from

Box 2. What does it take to become a trichromat?

Leaving aside arguments based upon selection, three things are required to become a trichromat:

- The gain of an additional opsin gene;
- The expression of this novel gene in a manner in which a unique chromatic signal can be produced;
- Having the neural circuitry to extract the colour information.

The evolutionary constraints arising from this fundamental logic of colour vision could help explain why primates are the only mammals to evolve trichromacy, and why routine trichromacy has not evolved more often in the primate lineage.

Gene mutation and duplication

The mutational mechanisms that could give rise to opsin functional diversification, and hence trichromacy, are straightforward. Opsins with novel spectral sensitivities can evolve by single point substitutions, and an increase in opsin gene number can occur by gene duplication (which is very common across the genome over evolutionary timescales [64]).

Gene expression

However, for a duplicated opsin gene to generate a novel chromatic signal in the retina, there must be differential expression of the genes in different cones. This is not a problem in allelic trichromacy, in which the different X-linked opsin alleles in a heterozygous female are expressed in different cones by virtue of random X-chromosome inactivation (Lyonization). Recently, an elegant experiment using transgenic mice suggests that differential expression of M and L genes in catarrhines follows a simple stochastic mechanism involving competition for the locus control region (LCR), and such a mechanism could automatically follow duplication [65]. However, this model requires a short distance between duplicated M and L genes, and does not apply in howlers, which have a larger duplication that includes the locus control region [13]. Whatever the precise mechanism of opsin gene expression, it is likely that many opsin duplications are selected against because the duplicated genes are not expressed in a manner that leads to a useful and novel chromatic signal.

Retinal circuitry of primates versus non-primates

Nonprimate mammals pool (i.e. sum) the outputs of several cone receptors before transmission to the brain, via retinal ganglion cells in the optic nerve. Pooling entails loss of spatial resolution, and also means that, were a new spectral type of photoreceptor to appear, it would not be possible to derive an additional chromatic signal without parallel modification of neural connections. In comparison to other mammals (Fig. Ia), primates have an additional class of retinal ganglion cell that receives its primary ('centre') input from a single cone cell (Fig. Ib). These so-called midget ganglion cells encode fine spatial detail and, in trichromatic primates, the red–green signal [66–68]. It might be that midget ganglion cells in primates first evolved to connect single cones to the brain for some purpose other than colour vision, perhaps to see fine patterns. This neural pathway then enabled the detection of separate M and L opsins when they appeared in primates, but is not present in other mammals.

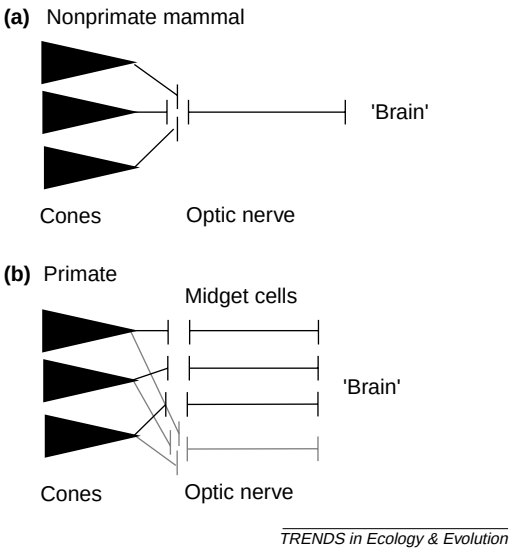

Fig. I.

orbit diameters and conclude that the common ancestor of anthropoid and prosimian primates was nocturnal, and hence unlikely to have been a trichromat. Accordingly, prosimians and anthropoids must have gained allelic trichromacy separately (Fig. 1).

DNA data indicate that the genetic divergence between New World primate alleles (2.6%) is considerably smaller than the divergence between Old World primate genes (6.1%) [16]. Hence, the New World primate alleles are likely to have arisen after the Old World gene duplication. However, once again gene conversion could have homogenized not only the two Old World genes, obscuring any ancestral allelic differences, but also the differences between the New World alleles. For example, evidence for a separate origin of the three New World alleles in different New World primate lineages from DNA sequence analysis is almost certainly attributable to gene conversion [22].

In summary, it seems probable that routine trichromacy in catarrhines and platyrrhines arose from independent tandem duplication events placing two allelic forms of the opsin gene on the same chromosome. Therefore, opsin gene polymorphism was probably present before the catarrhine–platyrrhine divergence. However, in principle at least, polymorphism in the opsin gene might have arisen independently, through point mutation, on more than one occasion. Such novel variants would be expressed in different cone cells through X-chromosome inactivation (LYONIZATION) when they occurred in the heterozygous form [7]. If neural mechanisms were already in place for heterozygous females to exploit different cone pigments and achieve trichromatic vision (Box 2), such polymorphisms would increase in a population through the process of heterozygous advantage whenever they were useful (e.g. in diurnal species). Alleles could be lost and gained through time as the environment and selective pressures changed during primate evolution. So what selective pressures might lead to the evolution of trichromacy, possibly many times, through different genetic routes?

Primate adaptation and selection for colour vision

There can be little doubt that trichromacy is advantageous because a duplicated gene is unlikely to survive and become fixed in the absence of positive selection. Similarly, the evolutionary persistence of multiple alleles at the opsin

locus in many lineages of New World primates is highly unlikely if such alleles were selectively neutral [23].

Allen's [1] idea that colour vision evolved for finding food remains popular [24–27]. However, primate diets vary widely, and hence the exact tasks on which selection is acting are controversial. In addition to frugivory, leaves are important foods for Old World anthropoids and howler monkeys and it might be that the need to locate edible leaves rather than fruit has been crucial in giving a selective advantage to trichromats (Box 3). But whether folivory exerted an additional selective force leading to routine trichromacy that is absent in other primate lineages, or is a secondary food resource exploited after the evolution of routine trichromacy, is difficult to say. It could be possible that selection toward routine trichromacy does involve some change in foraging behaviour [16,28].

Allen [1] proposed further that, once trichromacy evolved, it could be 'recruited' for other purposes. It is notable that many trichromatic primates use bright yellows and reds for intra-specific signaling; for example, the red sexual skin of ovulating female baboons and chimpanzees, the red on the nose and rump of mandrills and the bright orange coat of infants of many colobine species [29].

The puzzle of polymorphism

Polymorphism in the X-linked opsin gene is widespread in primates (Table 1), and provides an excellent example of different alleles being maintained in an apparent equilibrium by selection. However, the exact mechanism of this BALANCING SELECTION is not clear, and further questions are raised by the number and distribution of alleles among different species.

The simplest explanation for the maintenance of the polymorphism is through a consistent fitness advantage for heterozygous trichromatic females (HETEROSIS or overdominance). Under this model, a gene duplication leading to routine trichromacy would be strongly selected and expected to spread through the population. But if such functional duplications were to occur rarely (Box 2), then increasing the number of alleles would lead to an increased frequency of heterozygous females. With two opsin alleles, there would be 50% trichromatic females, with three alleles 67% and with four alleles 75% (under the Hardy–Weinberg equilibrium). However, most species of New World monkeys with opsin polymorphism have just three alleles (Table 1). Within this tri-allelic system, although heterozygous individuals probably have a higher fitness than do homozygotes, homozygous phenotypes are of roughly equal fitness. Compared with other situations with heterozygote advantage, this might be unusual, and could help explain the stability of allelic trichromacy. The number of useful alleles might also be limited by the available 'space' in the red–green spectrum between ~535 nm and 565 nm [30] (Box 1). Although this could be theoretically true, the relative closeness in wavelength of the common 556 nm and 563 nm alleles in callitrichines suggests that further alleles could be accommodated and that allelic 'saturation' has not been reached in most species.

An alternative possibility to simple heterozygote advantage is frequency dependence [7]. This possibility arises because there could be some situations in which dichromats have an advantage over trichromats. Human dichromats can detect patterns based on lightness that are indistinguishable to trichromats [31]. Dichromatic primates might therefore be better at detecting camouflaged predators or prey. The foraging of different phenotypes in visually distinct niches will then maintain both dichromats and trichromats in a population through frequency-dependent selection [7,32].

A final theoretical possibility is that balancing selection is acting not on the opsin locus but on a linked locus on the X chromosome. However, the maintenance of the opsin polymorphism in many lineages, over long periods of evolutionary time with recombination, makes this very unlikely.

How natural selection operates on traits such as colour vision in primates captures the interest of biologists from many fields, but often proves impossible to answer. Hypotheses such as those described here can be speculative and difficult to test in nature. However, the presence of dichromatic and trichromatic primates of the same species, and even the same sex, presents a unique opportunity for testing the models of balancing selection proposed by Mollon *et al.* nearly 20 years ago [7]. The relative foraging abilities of different colour vision phenotypes can be compared directly, both in captivity and in the wild. This is possible because an individual's colour vision

Box 3. Fruit or leaves?

Diurnal primates eat mainly fruit and young leaves, and it has long been argued that trichromatic colour vision is an adaptation for frugivory. The specific spectral tuning of the M and L photopigments can be explained if they are selected for finding fruit [2]. However, a difficulty with this hypothesis is that Old World monkeys are uniformly trichromatic, but not all eat ripe fruit. The colobines (Colobinae), which are a major subgroup in Africa and Asia, eat mainly leaves and unripe fruit. At the same time, trichromatic howlers, *Alouatta* spp., are among the most folivorous New World monkeys. Lucas and colleagues propose that primate trichromacy evolved primarily for selecting young nutritious leaves on a background of tougher mature leaves [3]. They argue that young leaves are detectable only by a red–green signal, whereas many fruits present a blue–yellow signal visible to dichromats [4]. Although this work shows that trichromacy is valuable for finding young nutritious leaves in the tropics, Sumner and Mollon present a strong case that the data do not distinguish between the folivory and frugivory hypotheses [69]. In addition, the claim that trichromacy is not important for finding fruit because of its absence in the New World where most species are not folivorous, is not substantiated. Trichromacy is observed in nearly all New World primate species and the existence of allelic trichromacy cannot be explained in the absence of or under relaxed selection. Trichromacy in primates benefits both frugivory and folivory [26]. Furthermore, the maximum wavelength of photopigments might not be set by the targets themselves, but by the background within which such targets are set (i.e. mature leaves) [26]. This might help to explain the similarity in spectral tuning of primate photopigments in both the Old and New Worlds in spite of the differences in primate diets.

phenotype can now be determined with reasonable accuracy from its genotype, by scoring the DNA nucleotides present at the amino acid sites that are crucial for spectral tuning. By combining behavioural ecology with population genetics in this way, it has been possible to show a foraging advantage for trichromatic over dichromatic marmosets *Callithrix geoffroyi*, based on food colour [33]. As opsin genotyping can be performed reliably using DNA in faeces of wild primates [34], many more studies attributing individual differences in foraging success to colour vision phenotype can be expected.

Colourblind monkeys and men

Finally, in contrast to the polymorphic New World monkeys, photopigment spectral tuning is remarkably uniform in the Old World anthropoids (Table 1), inviting speculation as to the uniform nature of selection acting upon the evolution of their opsin genes. However, humans are one notable exception. Approximately 8% of male Caucasians have colour vision defects, whereas such defects in other species appear to be very rare (e.g. 0.4% in male crab-eating macaques) [35–38]. Many humans have multiple copies of the M gene, but only the first M gene appears to be transcribed [39]. The frequency of multiple M copies appears to be much lower in other Old World primates [38]. Whilst the multiple M copies in humans might act to reduce the risk of eliminating the M gene completely during an unequal recombination event [11], they almost certainly increase the frequency of such events that then generate L/M hybrid genes [38]. Such hybrid genes result in anomalous trichromacy, but could increase in the population because the selective mechanisms operating on human colour vision are relaxed compared with other Old World primates (where, for example, group collection and pooling of food resources is less common than in humans and there is an increased dependence on fruit or leaves). In addition, colour vision defects could increase and spread in human populations through population bottlenecks and expansion events. For example, in the Pingelapese Islands of Micronesia, 5% of the population exhibits a very rare form of complete colour blindness, presumably because of a combination of a bottleneck effect and genetic isolation [40]. Such effects might not be so pronounced in most other primate species, in which large-scale migrations are rare and historical population sizes larger.

Prospects

Primates are unique among eutherian mammals in having trichromatic colour vision, and different groups of primates have achieved trichromacy through different genetic mechanisms. There is little dispute that trichromacy gives a selective advantage, and the direct link between genotype and phenotype makes it possible to demonstrate this. Two main issues remain unresolved. First, strong selection for trichromacy could have resulted in its evolution much earlier in the primate lineage than was previously thought, and challenges a long-standing belief that the ancestral primate was nocturnal. To elucidate the importance of trichromacy to early primates and help therefore to resolve the controversy surrounding the ancestral state of primates, we need to extend our understanding of the colour vision of prosimians.

Second, for primate species with allelic trichromacy, the demonstration of a foraging advantage for heterozygous females might prove to be a rare example of heterosis. Future studies should address the importance of colour vision in other aspects of primate life history; for example, does an increased ability to find coloured fruit increase the chances of survival and reproductive success of a trichromat in the wild? It remains to be shown whether there are advantages for dichromats (e.g. in finding camouflaged prey or detecting predators) and whether frequency dependence is important in the maintenance of polymorphism. Studies that combine both behavioural ecology and genetics will continue to provide a wealth of data for testing adaptionist hypotheses concerning the evolution of colour vision and other important physiological traits.

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