PERGAMON

Vision Research 38 (1998) 3299–3306

Vision Research

Molecular evolution of trichromacy in primates

David M. Hunt [a,*], Kanwaljit S. Dulai [a], Jill A. Cowing [a], Catherine Julliot [c], John D. Mollon [c], James K. Bowmaker [b], Wen-Hsiung Li [d], David Hewett-Emmett [d]

[a] *Department of Molecular Genetics, Institute of Ophthalmology, University College London, Bath Street, London EC1V 9EL, UK*
[b] *Department of Visual Science, Institute of Ophthalmology, University College London, Bath Street, London EC1V 9EL, UK*
[c] *Department of Experimental Psychology, University of Cambridge, Cambridge CB2 3EB, UK*
[d] *Human Genetics Center, School of Public Health, University of Texas, PO Box 20334, Houston, TX 77225, USA*

Received 10 July 1997; received in revised form 17 November 1997

Abstract

Although trichromacy in Old and New World primates is based on three visual pigments with spectral peaks in the violet (SW, shortwave), green (MW, middlewave) and yellow–green (LW, longwave) regions of the spectrum, the underlying genetic mechanisms differ. The SW pigment is encoded in both cases by an autosomal gene and, in Old World primates, the MW and LW pigments by separate genes on the X chromosome. In contrast, there is a single polymorphic X-linked gene in most New World primates with three alleles coding for spectrally distinct pigments. The one reported exception to this rule is the New World howler monkey that follows the Old World system of separate LW and MW genes. A comparison of gene sequences in these different genetic systems indicates that the duplication that gave rise to the separate MW and LW genes of Old World primates is more ancient than that in the howler monkey. In addition, the amino acid sequences of the two howler monkey pigments show similarities to the pigments encoded by the polymorphic gene of other New World primates. It would appear therefore that the howler monkey gene duplication arose after the split between New and Old World primates and was generated by an unequal crossover that placed two different forms of the New World polymorphic gene on to a single chromosome. In contrast, the lack of identity at variable sites within the New and Old World systems argues for the origin of the separate genes in Old World primates by the duplication of a single form of the gene followed by divergence to give spectrally distinct LW and MW pigments. In contrast, the similarity in amino acid variation across the tri-allelic system of New World primates indicates that this polymorphism had a single origin in New World primates. A striking feature of all these pigments is the use of a common set of substitutions at three amino acid sites to achieve the spectral shift from MW at around 530 nm to LW at around 560 nm. The separate origin of the trichromacy in New and Old World primates would indicate that the selection of these three sites is the result of convergent evolution, perhaps as a consequence of visual adaptation in both cases to foraging for yellow and orange fruits against a green foliage.

Keywords: Molecular evolution; Trichromacy; Primates

1. Introduction

Primates are unique amongst mammals in possessing trichromatic colour vision. Their trichromacy is based on three visual pigments with spectral peaks (λ_{max}) in the violet (shortwave, SW), green (middlewave, MW) and yellow–green (longwave, LW) regions of the spectrum. In Old World or catarrhine primates, the SW pigment is encoded by an autosomal gene and the MW and LW pigments by separate genes on the X chromosome [1,2]. In contrast, the trichromacy in most New World or platyrrhine primates is dependent on only two genes, an autosomal SW gene and a polymorphic X-linked LW/MW gene such that three (or more) pigments with differing λ_{max} values are encoded by different forms of the gene. Male monkeys combine the SW gene with just one of the set of polymorphic genes and so can generate only two cone pigments. They are therefore dichromats. In females with a different form (allele) of the LW/MW gene on each X chromosome, X-inactivation will ensure that only one allele is expressed per photoreceptor [3]. The presence of trichromacy in such females has been demonstrated behaviourally by Tovée et al. [4].

* Corresponding author. Tel.: +44 171 6086820; fax: +44 171 6086863; e-mail: d.hunt@ucl.ac.uk.

0042-6989/98/$19.00
PII: S0042-6989(97)00443-4

Visual pigments are composed of a protein moiety (opsin) that forms seven transmembrane α-helices. The chromophore in all mammalian pigments is 11-*cis*-retinal which is covalently bound via a protonated Schiff base to a lysine residue in the seventh transmembrane region. The spectral shifts between the pigments must be the result therefore of amino acid substitutions in the respective opsin proteins. On the basis of studies with two species of New World primates, the squirrel monkey and the tamarin, and with dichromatic human observers, Neitz et al. [5] proposed that the spectral shifts between these pigments are largely the result of amino acid substitutions at only three sites, 180 encoded by exon 3, and 277 and 285 encoded by exon 5. In each case, the substitution involved the gain of an hydroxyl-bearing amino acid in the longer-wave sensitive pigment. A similar conclusion was reached by Williams et al. [6] from studies of the common marmoset, with the additional suggestion that site 233 encoded by exon 4 may also be involved. These conclusions were essentially confirmed in a series of in vitro expression experiments with modified pigments generated by site-directed mutagenesis [7,8]. Substitutions at sites 180, 277 and 285 were found to contribute 7, 10 and 16 nm, respectively, to the LW to MW shift, with much smaller effects from other sites including 233.

Implicit in the original hypotheses of Neitz et al. [5] and Williams et al. [6] was that the mechanism of spectral tuning is the same in New and Old World primates, despite the sexual dimorphism of trichromacy in platyrrhines. It might therefore be supposed that the LW and MW genes of Old and New World primates have a common evolutionary origin. Since the mechanism of spectral tuning is essentially the same in both classes of primates, one hypothesis is that the two-gene system of Old World primates originated by unequal crossing-over between chromosomes carrying alleles encoding essentially LW and MW variants of the X-linked opsin gene, thus placing a separate LW and MW gene on to a single X chromosome. This hypothesis requires that the evolution of the polymorphic system of New World monkeys pre-dates the separation of the Old and New World primate lineages. The alternative hypothesis is that the trichromacy of New World primates is more recent and that the use of the same set of amino acid substitutions for the spectral tuning of the pigments is the result of convergent evolution.

The first molecular evidence for the latter hypothesis came from a study of the nucleotide sequence of intron 4 of the LW/MW opsin gene in two species of New World monkeys, the marmoset and squirrel monkey [9]. Since the different alleles show a high level of inter-species sequence divergence, the most parsimonious explanation is that the polymorphism arose separately in each New World species. More recently, an additional complication has arisen, namely the discovery of a two gene system in a New World primate, the howler monkey [10]. Is this evidence that the gene duplication in Old World primates preceded the catarrhine/platyrrhine split and that one of these genes was subsequently lost in other New World primate species? Initial sequencing of exon 5 of the howler monkey gene by Jacobs et al. [10] identified five sites at which nucleotide variation exists, with two of these following the pattern of variation in the polymorphic gene of other New World monkeys. However, the individual sequence of each gene was not reported and the sequencing was restricted to exon 5.

In this paper, we report the sequences of exons 3, 4 and 5 of the two genes in the howler monkey, *Alouatta seniculus*, and the equivalent regions of the three allelic forms of the single polymorphic gene in the capuchin monkey, *Cebus apella*. From comparisons of these sequences with those of the marmoset, *Callithrix jacchus* [6,11] and a number of Old World primates [1,2,12], we have been able to establish the most likely evolutionary origin of the duplicated genes in Old World primates and the howler monkey, and of the platyrrhine polymorphism.

2. Materials and methods

2.1. DNA samples and spectral phenotypes

DNA was isolated from blood samples taken from a single male howler monkey, *Alouatta seniculus*. The spectral phenotypes of the three capuchin monkeys, *Cebus apella*, were determined either by microspectrophotometry (550 nm animal) as described by Bowmaker et al. [13] or by the method of parvocellular recording (563 and 535 nm animals) as described by Yeh et al. [14]. DNA was extracted from liver samples by standard phenol–chloroform methods.

2.2. Amplification and sequencing of opsin gene fragments

The polymerase chain reaction (PCR) was used to amplify exons 3, 4 and 5 of the opsin genes as described by Dulai et al. [12]. Amplification of exon 3 fragments was achieved using the primer pair Op3+ (5′-ATCACAGGTCTCTGGTCTCTG-3′) and Op3− (5′-CTCCAACCAAAGATGGGCGG-3′), exon 4 fragments with primer pair Op4+ (5′-CACGGCCTGAAGACTTCATGC-3′) and Op4− (5′-CGCTCGGATGGCCAGCCACAC-3′), and exon 5 fragments with primer pair Op5+ (5′-GAATCCACCCAGAAGGCAGAG-3′) and Op5− (5′-ACGGGGTTGTAGATAGTGGCA-3′). Each reaction contained ca. 200 ng of template DNA, 200 ng of each primer, 1.5 mM $MgCl_2$, 0.2 mM each of dATP, dCTP, dGTP and dTTP,

0.25 units of *Taq* polymerase and reaction buffer in a final volume of 50 μl. Thirty-five cycles were used with an annealing temperature of 58°C, elongation temperature of 72°C, and denaturing temperature of 94°C. The products of the reaction were visualised by electrophoresis in a 1.5% low melting point agarose gel using a 0.09 M Tris–borate, 0.002 M EDTA buffer pH 8.0.

Amplified fragments were either TA cloned (Invitrogen) into pTAg plasmid vectors, or directly sequenced. All templates used for sequencing were cleaned through Centricon 100 columns prior to cycle sequencing. Approximately 400 ng of DNA template was used in conjunction with the ABI Prism™ Dye Terminator Cycle Sequencing Ready Reaction kit, and 3.2 pmol of each PCR primer. Cycle sequencing was performed on a Perkin Elmer 9600 PCR machine. The cycled products were loaded on to an ABI 373A sequencer and electrophoresed at 30 W for 12 h. Sequence data was analysed using the Sequence Navigator™ application.

2.3. Phylogenetic analysis

Pairwise divergence values (*p* values) for total nucleotides were used to generate a neighbour-joining tree [15] with the MEGA computer package [16]. Phylogenetic trees were generated by the neighbour-joining method of Saitou and Nei [15]. Support for internal branching was assessed by bootstrapping with 500 replicates.

3. Results and discussion

A cladistic classification of New World monkeys [17] is shown in Table 1. Only those species relevant to the present analysis are shown. Two main families are identified, the Atelidae, which includes the howler monkey, and the Cebidae, which is further divided into the subfamilies of the Cebinae, the Saimirinae, and the Callitrichinae.

Table 1
Cladistic classification of New World primates

Infraorder Platyrrhini
Superfamily Ceboidea
Family Atelidae
Subfamily Atelinae
Genus *Alouatta*: Howler monkey
Genus *Ateles*: Spider monkey
Family Cebidae
Subfamily Cebinae
Genus *Cebus*: Capuchin monkey
Subfamily Saimirinae
Genus Saimiri: Squirrel monkey
Subfamily Callitrichinae
Genus *Saguinus*: Tamarin
Genus *Callithrix*: Marmoset

Only genera relevant to this report are listed. From ref. [17].

Table 2
Amino acid substitutions at the major spectral tuning sites in the LW/MW opsin genes in Old and New World primates

		Amino acid sites		
	λ_{max} (nm)	180	277	285
Human LW	561	S	Y	T
MW	530	A	F	A
Chimpanzee LW	[a]	S	Y	T
MW	[a]	A	F	A
Gorilla LW	[a]	S	Y	T
MW	[a]	A	F	A
Diana monkey LW	566	S	Y	T
MW	531	A	F	A
Macaque LW	566	S	Y	T
MW	533	A	F	A
Talapoin monkey LW	564	S	Y	T
MW	533	A	F	A
Capuchin monkey	563	S	Y	T
	550	A	F	T
	535	A	F	A
Marmoset	563	S	Y	T
	556	A	Y	T
	543	A	Y	A
Howler LW	[a]	S	Y	T
Howler MW	[a]	A	F	A

λ_{max} values are from refs. [10,13].
[a] Precise λ_{max} values have not been obtained. Amino acid sequence of the LW and MW opsins would indicate that they are similar to human.

The LW/MW cone opsin gene has previously been analysed in the common marmoset [6,11], a member of the Callitrichinae. In this species, a similar pattern of amino acid substitutions is present at sites 180 and 285 to that of the LW and MW pigments of Old World primates but site 277 is invariant (summarised in Table 2). The sequences for a single member of the Saimirinae, the squirrel monkey *Saimiri sciureus*, and a second member of the Callitrichinae, the tamarin *Saguinus fuscicollis*, were reported by Neitz et al. [5]. However, the complete sequencing of the three alleles in the squirrel monkey [18] has identified discrepancies in the sequences presented by Neitz et al. [5] which appear to have arisen from contaminating human DNA. It is not possible therefore to use the data from Neitz et al. [5] in the present analysis.

In order to extend the analysis to include the Subfamily Cebinae, the LW/MW opsin genes in three male capuchin monkeys, *Cebus apella*, have been examined. The three monkeys selected represent the three spectral phenotypes with λ_{max} values at 563, 550 and 535 nm, respectively. A single male howler monkey, *Alouatta seniculus*, has also been analysed. The deduced amino acid sequences of exons 3, 4 and 5 are shown in Fig. 1, aligned with the equivalent human LW and MW se-

Exon 3

Human LW	ala	ile	ile	ser	trp	glu	arg	trp	leu	val	val	cys	lys	pro	phe	gly	asn	val	arg	phe 164
Human MW	.	.	.	.	.	.	.	.	met	.	.	.	.	.	.	.	.	.	.	.
Capuchin 563	-	-	-	-	-	-	-	-	.	.	.	.	.	.	.	.	.	.	.	.
Capuchin 550	-	-	-	-	-	-	-	-	.	.	.	.	.	.	.	.	.	.	.	.
Capuchin 535	-	-	-	-	-	-	-	-	.	.	.	.	.	.	.	.	.	.	.	.
Howler LW	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
Howler MW	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.

Human LW	asp	ala	lys	leu	ala	ile	val	gly	ile	ala	phe	ser	trp	ile	trp	ser	ala	val	trp	thr 184
Human MW	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	ala	.	.	.	.
Capuchin 563	.	.	.	.	.	.	.	.	val	.	.	.	.	.	.	.	.	.	.	.
Capuchin 550	.	.	.	.	.	.	.	.	val	.	.	.	.	.	.	ala	.	.	.	.
Capuchin 535	.	.	.	.	.	.	.	.	val	.	.	.	.	.	.	ala	.	.	.	.
Howler LW	.	.	.	.	.	.	.	.	val	.	.	.	.	.	.	.	.	.	.	.
Howler MW	.	.	.	.	.	.	.	.	val	.	.	.	.	.	.	ala	.	.	.	.

Human LW	ala	pro	pro	ile	188
Human MW	.	.	.	.	
Capuchin 563	-	-	-	-	
Capuchin 550	-	-	-	-	
Capuchin 535	-	-	-	-	
Howler LW	.	.	.	.	
Howler MW	.	.	.	.	

Exon 4

Human LW	gly	pro	asp	val	phe	ser	gly	ser	ser	tyr	pro	gly	val	gln	ser	tyr	met	ile	val	leu 223
Human MW	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
Capuchin 563	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
Capuchin 550	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
Capuchin 535	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
Howler LW	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
Howler MW	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.

Human LW	met	val	thr	cys	cys	ile	ile	pro	leu	ala	ile	ile	met	leu	cys	tyr	leu	gln	val	trp 243
Human MW	.	.	.	.	.	.	thr	.	.	ser	.	.	val	.	.	.	.	.	.	.
Capuchin 563	.	ile	.	.	.	phe	leu	.	.	gly	.	.	val	.	.	.	.	.	-	-
Capuchin 550	.	ile	.	.	.	.	leu	.	.	ser	.	.	val	.	.	.	.	.	-	-
Capuchin 535	.	ile	.	.	.	.	leu	.	.	ser	.	.	val	.	.	.	.	.	-	-
Howler LW	.	ile	.	.	.	phe	leu	.	.	gly	.	.	glu	.	.	.	.	.	.	.
Howler MW	val	ile	.	.	.	.	leu	.	.	ser	.	.	val	.	.	.	.	.	.	.

Human LW	leu	ala	ile	arg	ala	248
Human MW	.	.	.	.	.	
Capuchin 563	-	-	-	-	-	
Capuchin 550	-	-	-	-	-	
Capuchin 535	-	-	-	-	-	
Howler LW	.	.	.	.	.	
Howler MW	.	.	.	.	.	

Exon 5

Human LW	lys	glu	val	thr	arg	met	val	val	val	met	ile	phe	ala	tyr	cys	val	cys	trp	gly	pro 283
Human MW	.	.	.	.	.	.	.	.	.	.	val	leu	.	phe	.	phe	.	.	.	.
Capuchin 563	.	.	.	.	.	.	.	.	.	.	.	met	.	.	.	.	.	.	.	.
Capuchin 550	.	.	.	.	.	.	.	.	.	.	.	leu	thr	phe	.	.	.	.	.	.
Capuchin 535	.	.	.	.	.	.	.	.	.	.	.	val	thr	phe	.	.	.	.	.	.
Howler LW	-	-	.	.	.	.	.	.	.	.	.	met	.	.	.	.	.	.	.	.
Howler MW	-	-	.	.	.	.	.	.	.	.	.	ile	.	phe	.	.	.	.	.	.

Human LW	tyr	the	phe	phe	ala	cys	phe	ala	ala	ala	asn	pro	gly	tyr	ala	phe	his	pro	leu	met 303
Human MW	.	ala	.	.	.	.	.	.	.	.	.	.	.	.	pro	.	.	.	.	.
Capuchin 563	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
Capuchin 550	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
Capuchin 535	.	ala	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
Howler LW	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
Howler MW	.	ala	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.

Human LW	ala	ala	leu	pro	ala	tyr	phe	ala	lys	ser	313
Human MW	.	.	.	.	.	phe	.	.	.	.	
Capuchin 563	.	.	.	.	.	.	.	.	.	.	
Capuchin 550	.	.	.	.	.	.	.	.	.	.	
Capuchin 535	.	.	.	.	.	.	.	.	.	.	
Howler LW	.	.	.	.	.	.	.	.	.	.	
Howler MW	.	.	.	.	.	.	.	.	.	.	

Fig. 1. Amino acid sequence encoded by exons 3, 4 and 5 of the LW/MW genes of the capuchin monkey, *Cebus apella*, and the howler monkey, *Alouatta seniculus*, aligned with the equivalent human LW and MW sequences [1]. Identical residues are indicated by a dot and missing data by a dash. The three exons of the howler monkey are identified as LW or MW on the basis of similarity of amino acid sequence to those of the marmoset and capuchin opsins.

Table 3
Percent nucleotide divergence of exons 3, 4 and 5 of primate LW/MW opsin genes

		New World primates					Old World primates
		Capuchin		Marmoset		Howler	
	λ_{max}	550	535	556	543	MW	MW
New World primates							
Capuchin	563	2.8	2.8				
	550		1.9				
Marmoset	563			2.3	3.5		
	556				2.3		
Howler	LW					2.7	
Old World primates							
Chimpanzee	LW						8.3
Human	LW						4.7
Gorilla	LW						5.6
Diana	LW						5.2
Macaque	LW						7.1
Talapoin	LW						5.9

quences. Only a single gene was found in each of the capuchin monkeys whereas, consistent with the report of Jacobs et al. [10], two distinct genes were identified in the howler monkey. Each of the three exons sequenced in this latter species is identified as either LW or MW on the basis of similarity of amino acid sequence with those of the marmoset and capuchin opsins. This does not take into account the possibility of alternative combinations of the three exons as seen in humans [19], although such a possibility does not invalidate the following analysis.

In the capuchin monkey, the substitutions at the major tuning sites follow exactly that reported for Old World primates [1,2,7,8]. The 563 and 550 pigments differ for ser180ala and tyr277phe substitutions, and the 550 and 535 pigments for thr285ala substitution. The same pattern is again seen in the howler monkey with substitutions at all three sites differentiating the putative LW and MW pigments. It is likely therefore that the capuchin monkey and the howler monkey use the same three sites as Old World primates for the spectral tuning of these cone pigments.

3.1. Divergence of opsin genes

In order to obtain a relative estimate of the antiquity of the separate genes in Old World primates and in the howler monkey, and the polymorphism in the other New World monkeys, we have determined the within-species nucleotide divergence between genes or alleles. As shown in Table 3, the divergence between the LW and MW genes of six species of Old World primates [12] ranges from 4.7% in human to 8.3% in chimpanzee. In contrast, the genes of the howler monkey show a much lower substitution rate of only 2.7%. Evidence for gene conversion between the LW and MW genes of Old World primates has been reported [2,20,21] and this will have the effect of reducing the rate of divergence. The howler monkey genes may also be subject to conversion, although in the absence of an independent estimate of the antiquity of the duplication, this cannot be assessed. There is however no reason to suppose that the effect of gene conversion would be greater in the howler monkey than in Old World primates. The greater divergence of the LW and MW genes in Old World primates than in the howler monkey indicates therefore that the gene duplication in the Old World primate lineage pre-dates that in the howler monkey. The duplication in Old World primates is thought to have occurred after the separation of the New World monkey lineage but at the base of the Old World primate lineage since it is present in all species of Old World monkeys so far examined. In contrast, the duplication in the howler monkey would appear to be limited to this genus since we have preliminary evidence that it is not present in a closely-related species, the spider monkey *Ateles geoffroyi*, another member of the family Atelidae.

A phylogenetic tree based on total nucleotide substitutions is consistent with this interpretation (Fig. 2). The main Old and New World primate lineages are clearly apparent with separate LW and MW genes in Old World primates appearing after the Old/New World branch point. In contrast, the howler monkey duplication is placed within the New World primate clade, after the generation of the polymorphic forms of the gene in the marmoset and capuchin monkey. The branch pattern also indicates that the polymorphism of New World primates arose after the split in the primate lineage; this would imply that the ancestral anthropoid

was a dichromat. Within the New World monkey clade, the polymorphisms in the marmoset and capuchin monkey would appear to have a separate origin. However, the branches within this region of the tree are not well supported by bootstrap confidence values and, as discussed later, the pattern of amino acid substitutions encoded by exon 4 across the three alleles would argue for a single origin of the New World primate polymorphism. The placement of the capuchin 550 and 535 alleles into a separate branch from the marmoset 556 and 543 may be the result of the recruitment of site 277 to the spectral shift of the capuchin pigments.

3.2. Origin of LW/MW opsin gene polymorphism/duplication in primates

The nucleotide divergence between the allelic forms of the LW/MW opsin gene in the marmoset and capuchin monkey ranges from 1.9 to 3.5%, with an average value of 2.6%. This is similar to the divergence between the separate howler monkey genes (2.7%) and considerably less than the divergence between the Old World primate genes (average of 6.1%), again indicating that the Old World primate gene duplication occurred before the generation of the polymorphism in New World monkeys. A non-quantifiable factor when considering divergence between alleles is however the extent of homogenisation by inter-allelic recombination; the polymorphism may therefore be much older and pre-date the Old and New World primate split.

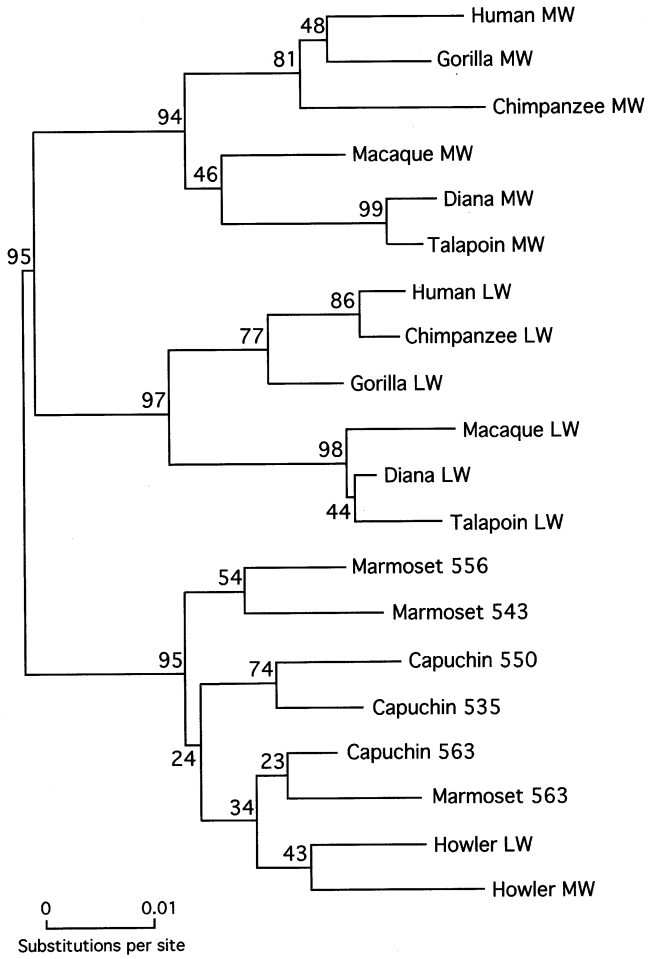

Fig. 2. Phylogenetic tree generated from nucleotide divergence of exons 3, 4 and 5 of the LW/MW opsin genes of Old and New World primates. The tree was generated by the neighbour-joining method [15]. The length of each branch reflects the evolutionary distance between genes or alleles. The scale bar is calibrated in substitutions per site. The bootstrap confidence values based on 500 replicates are shown for each branch.

As an alternative approach to this problem of whether the separate LW and MW genes of Old World primates arose from a pre-existing polymorphism, we have compared the amino acid sequences of the marmoset, capuchin and howler monkey genes with those of the great apes and cercopithecoid monkeys [2,12,22]. All sites that differ either between the LW and MW opsins of a majority of the great apes and cercopithecoid monkeys, or vary in the three New World primate species, are listed in Table 4. A total of 20 sites fall into these two categories and, except for the three major tuning sites, only position 173 shows an identical substitution of either isoleucine or valine in Old World primate MW genes and in the marmoset alleles. For all other sites, where the Old World primate LW and MW sequences differ, the site is either non-variant in New World monkeys or shows a different substitution. Conversely, where the New World primate sequences differ, the site is either non-variant in Old World monkeys or again shows a different substitution. In other words, if the tuning sites 180, 277 and 285 are discounted, there are essentially no similarities in amino acid substitutions between the separate genes of Old World primates and the polymorphic/duplicate genes of New World primates. Such a situation would not be expected if the Old World system evolved from a pre-existing polymorphism. The more parsimonious explanation is therefore that the two systems evolved quite separately and that the use of the same tuning sites is the result of convergent evolution. Convergent evolution would appear to be relatively common in visual pigments and may reflect the paucity of sites that can be altered to provide spectral shifts and yet be totally compatible with normal pigment function. There is evidence of the use of the same sites in, for example, the rod opsins of deep-sea and freshwater species of fish [23,24] and in fish cone pigments [25].

Amongst the New World monkeys, amino acid substitutions at sites 229 and 233 show a similar pattern across the three alleles of the marmoset and capuchin monkey and between the separate genes of the howler monkey. Since site 229 has not been implicated in spectral tuning and substitution at site 233 has only a 1–3 nm effect [8], it would seem unlikely that common

Table 4
Amino acid substitutions in the LW/MW genes of Old and New World primates

	Exon 3						Exon 4						Exon 5							
	153	171	173	174	178	**180**	224	225	229	230	233	236	274	275	276	**277**	279	**285**	298	309
Old World primates																				
Great ape LW	L	I/V	I	A	I	**S**	M	I/V	I	I	A	M/V	I	F	A	**Y**	V	**T**	A	Y
Great ape MW	M	V	I	A/V	I/V	**A**	M	I/V	I	T	S	V	V	L	A	**F**	F	**A**	P	F
Cercopithecoid LW	L	V	I	A	I	**S**	M	V	I	I	A	M	I	F	A	**Y**	V	**T**	A	Y
Cercopithecoid MW	M	V	I/V	A/V	I/V	**A**	M	V	I	T	T	V	F	L	A	**F**	F	**A**	A	Y
New World primates																				
Capuchin 563	L	V	V	A	I	**S**	M	F	F	L	G	V	I	M	A	**Y**	V	**T**	A	Y
Capuchin 550	L	V	V	A	I	**A**	M	I	I	L	S	V	I	L	T	**F**	V	**T**	A	Y
Capuchin 543	L	V	V	A	I	**A**	M	I	I	L	S	V	I	V	T	**F**	V	**A**	A	Y
Marmoset 563	L	V	V	A	I	**S**	M	I	F	L	G	V	I	V	A	**Y**	V	**T**	A	Y
Marmoset 556	L	V	I	A	I	**A**	M	I	F	L	S	V	I	V	A	**Y**	V	**T**	A	Y
Marmoset 543	L	V	I	A	I	**A**	M	I	I	L	S	V	I	V	A	**Y**	V	**A**	A	Y
Howler LW	L	V	V	A	I	**S**	M	I	F	L	G	E	I	M	A	**Y**	V	**T**	A	Y
Howler MW	L	V	V	A	I	**A**	V	I	I	L	S	V	I	I	A	**F**	V	**A**	A	Y

The major tuning sites are shown in bold. The great ape sequences are based on human [1], gorilla and chimpanzee [12,22], and the cercopithecoid monkeys on the diana monkey, the patas monkey, the talapoin monkey, the spot-nose monkey, the African green monkey and the macaque [2,12].

substitutions at these sites are critical for λ_{max} determination. This argues therefore for a single origin of the polymorphism in platyrrhine primates and, in direct contrast to the situation in Old World primates, for the origin of the separate LW and MW genes in the howler monkey from an unequal crossover between two different variants of the polymorphic gene. The alternative explanation that New World LW and MW pigments require phe229/gly233 and ile229/ser233 respectively for normal function and that convergent evolution is again involved would seem less likely since this would require selection of these residues at these two sites on three separate occasions.

It is generally considered that the trichromacy of primates is an adaptation to foraging for yellow and orange fruits amongst green foliage [26]. The simplest explanation for the maintenance of the polymorphism in New World primates is heterozygous advantage of trichromacy in females. The separate gene duplications in Old World primates and in the howler monkey indicate however that such events may not be uncommon. In this case, the non-fixation of such a duplication would require some selective advantage from the presence of dichromatic and trichromatic observers in the population, perhaps in foraging for different food sources. Fixation of a duplication may require therefore a change in foraging behaviour.

References

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

[2] Ibbotson RE, Hunt DM, Bowmaker JK, Mollon JD. Sequence divergence and copy number of the middle- and long-wave photopigment genes in Old World monkeys. Proc R Soc B 1992;247:145–54.

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

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

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

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

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

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

[9] Shyue S-K, Hewett-Emmett D, Sperling HG, Hunt DM, Bowmaker JK, Mollon JD, Li W-H. Adaptive evolution of color vision genes in higher primates. Science 1995;269:1265–7.

[10] Jacobs GH, Neitz M, Deegan JF, Neitz J. Trichromatic colour vision in New World monkeys. Nature 1996;382:156–8.

[11] Hunt DM, Williams AJ, Bowmaker JK, Mollon JD. Structure and evolution of the polymorphic photopigment gene of the marmoset. Vis Res 1993;33:147–54.

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

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

[14] Yeh T, Lee BB, Kremers J, Cowing JA, Hunt DM, Martins PR, Troy JB. Visual responses in the lateral geniculate nucleus of dichromatic and trichromatic marmosets (*Callithrix jacchus*). J Neurosci 1995;15:7892–904.

[15] Saitou N, Nei M. The neighbor-joining method: A new method for reconstructing phylogenetic trees. Mol Biol Evol 1987;4:406–25.

[16] Kumar S, Tamura K, Nei M. MEGA: Molecular Evolutionary Genetics Analysis, version 1.01. Pennsylvania State University, PA, 1993.

[17] Schneider H, Schneider MPC, Sampaio ML, Harada ML, Stanhope M, Czelusniak J, Goodman M. Molecular phylogeny of the New World Monkeys (Platyrrhini, Primates). Mol Phylogenet Evol 1993;2:225–42.

[18] Shyue S-K, Boissinot S, Schneider H, et al. Molecular genetics of spectral tuning in New World monkey color vision. J Mol Evol 1998, in press.

[19] Sanocki E, Lindsey DT, Winderickx J, Tellet DY, Deeb SS, Motulsky AG. Serine/alanine amino acid polymorphism of the L and M cone pigments: effects on Rayleigh matches among deuteranopes, protanopes and color normal observers. Vis Res 1993;33:2139–52.

[20] Balding DJ, Nichols RA, Hunt DM. Detecting gene conversion: primate visual pigment genes. Proc R Soc B 1992;249:275–80.

[21] Zhou Y-H, Li W-H. Gene conversion and natural selection in the evolution of X-linked color vision genes in higher primates. Mol Biol Evol 1996;13:780–3.

[22] Deeb SS, Jorgensen AL, Battisti L, Iwasaki L, Motulsky AG. Sequence divergence of the red and green visual pigments in great apes and humans. Proc Natl Acad Sci USA 1994;91:7262–6.

[23] Hunt DM, Fitzgibbon J, Slobodyanyuk SJ, Bowmaker JK. Spectral tuning and molecular evolution of rod visual pigments in the species flock of Cottoid fish in Lake Baikal. Vis Res 1996;36:1217–24.

[24] Hope AJ, Partridge JC, Dulai KS, Hunt DM. Mechanisms of wavelength tuning in the rod opsins of deep-sea fishes. Proc R Soc B 1997;264:155–63.

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

[26] Mollon JD. "Tho' she kneel'd in that place where they grew...". The uses and origins of primate colour vision. J Exp Biol 1989;146:21–38.