# Chapter 7
# Polymorphic Color Vision in Primates: Evolutionary Considerations

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## Abbreviations

| | |
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| $\lambda_{max}$ | Wavelength of maximal absorbance |
| cDNA | Complementary DNA |
| ERG | Electroretinogram |
| LCR | Locus control region |
| M/LWS | Middle to long wavelength-sensitive |
| MSP | Microspectrophotometry |
| PCR | Polymerase chain reaction |
| RH1 | Rhodopsin |

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| RH2 | Rhodopsin-like |
| SWS1 | Short wavelength-sensitive type 1 |
| SWS2 | Short wavelength-sensitive type 2 |

## 7.1 Color Vision and Opsins

Color vision is based on the ability to discriminate light by differences in wavelength (or hue). At least two different spectral types of photoreceptors in the retina are necessary to compare signals from these wavelengths. Generally speaking, the number of discriminable colors increases as the number of spectrally distinct photoreceptors increases and as the spectral overlap among them is reduced (Vorobyev 2004). However, this does not always hold true under dim light conditions. It should also be noted that some colors may be more important than others and that it may not even be necessary to perceive certain colors, depending on the ecological requirements for a given animal (Vorobyev 2004).

Vertebrate retinas contain two types of visual photoreceptor cells: rods and cones. Rods allow dim-light vision and cones allow daylight and color vision. Photosensitive molecules, called visual pigments, are located in the outer segments of these cells. A visual pigment consists of a protein moiety, opsin, and a chromophore, either 11-*cis* retinal or 11-*cis* 3,4-dehydroretinal (vitamin $A_1$ or $A_2$ aldehyde, respectively) (Nathans 1987). Opsins in general are retinal-mediated light-sensing proteins found in a variety of organisms from bacteria to vertebrates. These proteins have various visual and nonvisual functions. The vertebrate and invertebrate opsins belong to a multigene superfamily of the G-protein-coupled receptors (GPCR), which commonly form a characteristic seven-transmembrane structure. The chemosensory receptors, such as odor, taste, and pheromone receptors, also belong to the GPCR gene families (see also Chaps. 4–6). Amino acid sequences of the opsins modulate absorption spectra of the chromophore. Absorption spectra of visual pigments are bell shaped when plotted against wavelengths. The wavelength where peak absorbance occurs is called the lambda max, "$\lambda_{max}$." Given the uniformity of the absorption curve, the $\lambda_{max}$ is commonly used to represent the whole absorption spectra of a visual pigment.

In diurnal birds, reptiles, and lungfishes, colored oil droplets, located in cones, further modulate absorption spectra of the cones as colored filters (Walls 1942; Robinson 1994). These retinal filters reduce the overlap in sensitivity between spectrally adjacent cones and hence increase the number of discriminable colors (Govardovskii 1983). Thus, opsins, chromophores, and oil droplets together shape the spectral properties of cones. However, the A2 type chromophore is not used in most terrestrial vertebrates, and oil droplets are absent in many groups of animals (Lythgoe 1979; Goldsmith 1990). Thus, opsins play a universal and pivotal role in evolution of color vision. In addition, a major advantage to molecular evolution studies that focus on opsins lies in the feasibility of functional assays of these proteins, coupled with site-directed mutagenesis, by transfection of opsin cDNAs to cultured cells, reconstitution of functional photopigments in vitro with a chromophore, and spectral measurement of the purified pigments (Yokoyama 2000b).

For the evolution of color vision, animals need (1) a set of spectrally differentiated cone opsins, (2) the genetic mechanisms that allow the opsins to be expressed in different cones, (3) the neural mechanisms that enable the comparison of signals from the different spectral classes of cones and the extraction of color information, and (4) natural selection to promote and maintain a particular color vision status. Perhaps the most commonly endorsed interpretation of human trichromacy would still be the simple one that trichromacy is universally superior to dichromacy for any visual task, and that the dichromatic and vision-polymorphic animals may eventually acquire routine trichromacy, if the necessary events were to occur, that is, duplication and divergence of opsin genes leading to fixation of two spectrally different opsin genes. However, recent studies indicate a paradigm shift toward the view that the adaptive value of trichromacy is conditional rather than universal, depending on the specific ecological demands on animals in their environments. The question does remain about what exactly these conditions are. Because of the wide variation of color vision both within and between species, New World monkeys are excellent subjects for study of the utility of color vision in natural environments, which will help us to elucidate the selective advantage of being a trichromat or dichromat.

In the following section, we first introduce the current state of knowledge of variation in vertebrate visual opsins, and then shift our main focus to primates, covering all four requisites for the evolution of color vision just listed.

## 7.2 Visual Opsins in Vertebrates

The visual opsins in vertebrates are classified into five phylogenetic types: RH1 (rhodopsin or rod opsin), and four cone opsins [RH2 (rhodopsin-like, or green), SWS1 (short wavelength-sensitive type 1, or ultraviolet-blue), SWS2 (short wavelength-sensitive type 2, or blue), and M/LWS (middle to long wavelength-sensitive, or red–green)] (Yokoyama 2000a). It is well established that these five types were present in the common ancestor of all vertebrates, including jawless fish (Yokoyama 2000a; Collin et al. 2003, 2009; Davies et al. 2009). Thus, early vertebrates could already have had four-dimensional color vision (tetrachromacy). Many fish are known to possess a rich repertoire of visual opsins, including two or more opsin subtypes within the five types: for example, zebrafish (*Danio rerio*) have nine visual opsin genes including spectrally distinct two M/LWS and four RH2 opsin subtypes (Chinen et al. 2003). Many species of birds and reptiles retain one each of the four cone opsin types (and a rod opsin) and are tetrachromatic in color vision (Ebrey and Koutalos 2001). In contrast, mammals are considered to have lost RH2 and either the SWS2 (placental mammals and marsupials) or SWS1 (monotremes) opsin gene in a nocturnal ancestor that lived during the Mesozoic Period (Ahnelt and Kolb 2000; Davies et al. 2007). Extant placental mammals are basically dichromatic with only SWS1 and M/LWS as cone opsins besides a rod opsin (Jacobs 1993). Placental mammals use only 11-*cis* retinal as a chromophore, and all spectral variations of their visual pigments are caused by the opsins. Primates are the only exception among placental mammals in attaining trichromatic vision by diversifying the M/LWS opsin gene through either gene duplication or allelic diversification (Jacobs 1999).

Shadows generally yield strong variation in the intensity of illumination, but comparison of chromatic signals from different spectral types of cones provides a value that remains constant across different levels of illumination intensity (Foster and Nascimento 1994). Thus, color vision is especially useful for object detection in conditions of patchy and changing illumination or against a dappled background. In addition, color facilitates the identification of an object by its surface reflectance irrespective of spectral distribution of the illuminant, the phenomenon known as color constancy (Pokorny et al. 1991). Patchy and spectrally varying illumination is common in shallow water and in forests, and hence these are the places where color vision would be strongly selected for and be diversified most dramatically (Vorobyev 2004). Appearance of the four cone opsin types in early vertebrates before the divergence of jawed and jawless forms (Yokoyama 2000a; Collin et al. 2003, 2009; Davies et al. 2009) is intriguing in this context. Early vertebrates could already have had tetrachromatic color vision in their shallow aquatic habitat in the early Cambrian, approximately 540 million years ago (Maximov 2000). This idea also could explain why current fish are so varied and rich in the visual opsin repertoire, presumably reflecting their evolutionary adaptation to diverse aquatic light environments (Levine and MacNichol 1982). Similarly, in terms of the evolution of primate color vision, forest light may have been a key factor that prompted reclamation of the third opsin in primates, enabling their trichromacy (Mollon 1989).

## 7.3 Variation in Color Vision and Visual Opsin Repertoire Among Primates

### *7.3.1 Nomenclature*

Conventionally, the primate SWS1 opsin is called the "blue" or "S" opsin, with $\lambda_{max}$ at around 420–430 nm. The longer-wave subtype of the M/LWS opsin ($\lambda_{max}$ at around 560 nm) is often called the "red" or "L" opsin, and the shorter-wave subtype ($\lambda_{max}$ at around 530 nm) is called the "green" or "M" opsin. In the case of New World monkeys, in which three M/LWS opsin alleles are often found, alleles are called by their $\lambda_{max}$ values or by conventional color names, such as red, yellow (or orange), and green, or by the abbreviations "L," "A" ("A" stands for "anomalous" in human sense) (or "I"; "I" stands for "intermediate"), and "M." It should be noted that the color name does not necessarily match the corresponding color of the pure light of the $\lambda_{max}$: for example, the 560-nm light, a typical $\lambda_{max}$ value of "red" opsins, appears yellowish to trichromatic human observers.

### *7.3.2 Variation of Color Vision in Primates*

Among primates, variation in number and spectral properties of the M/LWS and SWS1 opsins results in marked variation in color vision (Fig. 7.1). Routine trichromacy

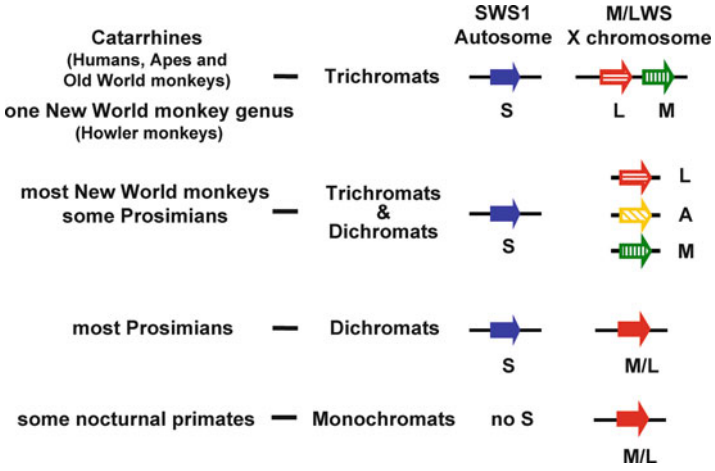


**Fig. 7.1** Variation in color vision and visual opsin repertoire among primates. Catarrhines and howler monkeys have L and M opsin genes (as the M/LWS, middle to long wavelength-sensitive or red-green class of opsin gene) on the same X chromosome and the S opsin gene (as the SWS1, short wavelength-sensitive type 1 or ultraviolet-blue class of opsin gene) on an autosome and are therefore uniformly trichromatic in color vision. Many New World monkeys and some prosimians have two or more M/LWS opsin alleles of a single X-chromosomal locus in addition to the autosomal S opsin gene and are polymorphic in color vision, consisting of both trichromats and dichromats. Three alleles are shown and labeled as L, A, and M in this figure. Most prosimians have a single M/LWS opsin locus (labeled M/L) with no allelic variation in addition to the autosomal S opsin gene and are uniformly dichromatic. Some nocturnal primates are monochromatic as a result of the loss of the functional S opsin gene

is attained by a gene duplication of the M/LWS opsin on the X chromosome and by spectral differentiation of the resulting L and M opsins in catarrhines (humans, apes, and Old World monkeys) (Nathans et al. 1986; Jacobs 1996). A similar gene duplication is reported for howler monkeys (*Alouatta*), a genus of New World monkeys (Jacobs et al. 1996a), although this was attained by an independent evolutionary event.

Color vision polymorphism, that is, a mixed population of dichromats and trichromats resulting from allelic variation of the X-linked M/LWS opsins (Fig. 7.2), has been documented in many species of New World monkeys (Fig. 7.3) (Jacobs 1998). In this system, females are either trichromatic or dichromatic, whereas males are all dichromatic. A wide variation of L–M opsin allelic composition occurs among the vision-polymorphic New World monkeys, ranging from diallelic, seen typically in *Ateles* (spider monkeys) and *Lagothrix* (woolly monkeys), up to pentallelic, reported for *Callicebus moloch* (dusky titi monkeys) (Jacobs and Deegan 2001, 2005; Talebi et al. 2006; Jacobs 2007). Similar allelic variation is reported for a few species of diurnal or cathemeral prosimians (Tan and Li 1999; Heesy and Ross 2001; Jacobs et al. 2002; Veilleux and Bolnick 2009).

Uniform dichromacy is considered a norm in many prosimians with a monomorphic M/LWS opsin and an SWS1 opsin, as is found in most placental mammals. Finally, monochromacy resulting from the loss of a functional SWS1 opsin has been documented in some nocturnal species, notably lorisiform prosimians and owl monkeys (*Aotus*), a genus of New World monkeys that is the only nocturnal simian

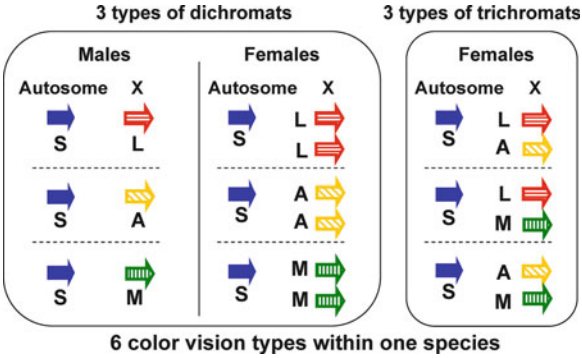


**Fig. 7.2** Color vision polymorphism of New World monkeys. Typically, three alleles are found in the X-linked M/LWS opsin gene, here labeled L, A, and M. Males have only one X chromosome and are therefore obligate dichromats, having a single M/LWS opsin allele on the X chromosome and the single autosomal S opsin gene. However, there are three types of dichromatic males within the same species, each having different M/LWS allele types. If a female has the same M/LWS opsin allele on both X chromosomes, she is also dichromatic, as are the males. If a female has two different M/LWS alleles, she is a trichromat. There are three types of trichromatic females in the species because three heterozygote combinations are possible in a triallelic M/LWS system. In total, six different color vision phenotypes can exist in one species if there are three M/LWS alleles

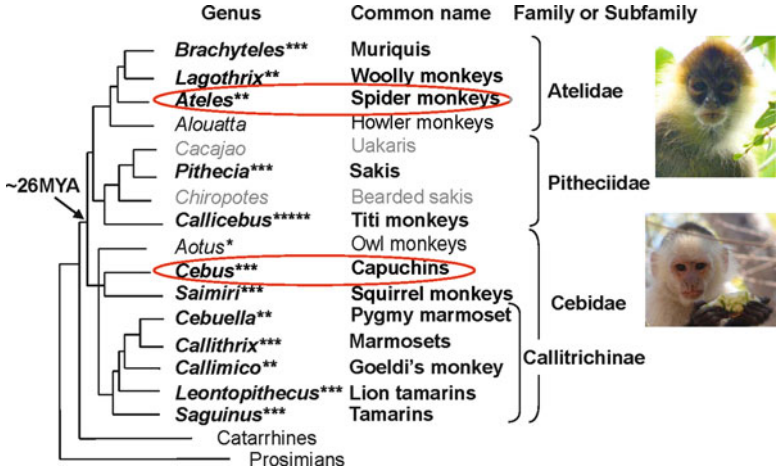


**Fig. 7.3** Phylogenetic distribution of color vision polymorphism among New World monkeys. Polymorphism has been reported for the genera indicated with *boldfaced letters*. The number of *asterisks* indicates the number of alleles reported for the genus (Jacobs 1998; Jacobs and Deegan II 2001; Surridge and Mundy 2002; Jacobs and Deegan 2003; Jacobs and Deegan 2005; Talebi et al. 2006). Spider monkeys and capuchins have been targeted in our field study and are *circled* with associated photographs. Howler monkeys (*Alouatta*) are regarded as uniformly trichromatic and owl monkeys (*Aotus*) as monochromatic. Color vision of monkeys indicated with *gray letters* (*Cacajao* and *Chiropotes*) is not known. (The phylogenetic tree is after Schneider 2000)

(catarrhines and New World monkeys) (Jacobs et al. 1996b; Kawamura and Kubotera 2004; Tan et al. 2005). An intact copy or multiple pseudogene copies of M/LWS opsin gene are present on the Y chromosome of owl monkeys in addition to the original X-linked one, but their functional significance remains unknown (Kawamura et al. 2002; Nagao et al. 2005).

Among routinely trichromatic catarrhines, humans constitute a notable exception. Approximately 3–8% of males have "color vision defects," mainly because of unequal meiotic recombination between L and M opsin genes (Deeb 2006). The L and M opsin genes are highly similar in nucleotide sequence (~96% identity) and are closely juxtaposed (Nathans et al. 1986). The recombination results in an L–M hybrid gene if the crossover point lies within a gene region. If the crossover is in an intergenic region, it results in gene deletion in one chromosome and duplication in the other. Many humans have multiple copies of the M opsin gene in the M/LWS opsin gene array, wherein the most upstream gene is typically L and the others are M. Only the upper two genes are expressed, and when a hybrid gene occupies either position, it causes anomalous trichromacy (Hayashi et al. 1999). When there is only one M/LWS opsin gene on an X chromosome or when the two positions are occupied by the same genes, this causes dichromacy (red–green colorblindness: more specifically, protanope when L is lost, and deuteranope when M is lost). Color vision defects caused by these mutations in the M/LWS opsin gene include both anomalous trichromacy and dichromacy. These phenotypes are typically found in men because women have two X chromosomes and thus are more likely to have a "normal" gene array in either one. There are rare cases of individuals, irrespective of sex, who have no functional blue cones (tritanopes, <1:10,000) because of mutations in the S opsin gene on chromosome 7 (Sharpe et al. 1999).

Compared to humans, the incidence of color vision defects in non-human catarrhines is low (Onishi et al. 1999; Jacobs and Williams 2001; Terao et al. 2005). Among 744 male long-tailed macaques (*Macaca fascicularis*) examined, only 3 were found to have a single hybrid M/LWS opsin gene and to be dichromats (Onishi et al. 1999; Hanazawa et al. 2001). Among 58 male chimpanzees (*Pan troglodytes*), 1 was found to have a hybrid gene in addition to one normal M opsin gene on the X chromosome and to be an anomalous (protanomalous) trichromat (Saito et al. 2003; Terao et al. 2005). Thus, frequencies of color vision deficiencies in male long-tailed macaques and male chimpanzees can be calculated to be about 0.4% and about 1.7%, respectively. These frequencies could be overestimated because no defects were found in 455 male monkeys from other macaque species (Onishi et al. 1999) and the chimpanzees examined were from limited numbers of breeding colonies (Terao et al. 2005). Other researchers have reported an absence of color vision defects in Old World monkeys and apes (Jacobs and Williams 2001). Multiple copies of M opsin genes are likely to increase the frequency of unequal recombination events. Although multiple M copies are found in 66% of human (Caucasian) males (Drummond-Borg et al. 1989), they were found in only 5% of 130 male long-tailed macaques (Onishi et al. 1999, 2002) and 6% of the 58 male chimpanzees (Terao et al. 2005). The low incidence of the multiple M copies may partly explain the low incidence of color vision defects in these primates.

However, other studies report that multiple copies are common among Old World monkeys and apes (Ibbotson et al. 1992; Dulai et al. 1994).

### 7.3.3 Molecular Evolution of the L–M Opsin Gene in Primates

Mutagenesis studies indicate that the $\lambda_{max}$ of the vertebrate M/LWS opsins can be predicted from the amino acid composition at the three sites, 180, 277, and 285 (the residue numbers hereafter follow those in the human L opsin), together with two additional sites, 197 and 308 ("five-site rule") (Yokoyama and Radlwimmer 1998, 1999; Yokoyama et al. 2008). The residue 180 is encoded in exon 3, 197 in exon 4, and 277, 285, and 308 in exon 5. Among primate M/LWS opsins, however, the residues 197 and 308 are not varied and are irrelevant to spectral differences among them. Therefore, the five-site rule can be reduced to the "three-site rule" in primates, wherein amino acid changes from Ser to Ala at site 180 (denoted Ser180Ala), Tyr277Phe, and Thr285Ala shift the $\lambda_{max}$ values by −7, −8, and −15 nm, respectively, and the reverse amino acid changes cause opposite spectral shifts to the same extent in a nearly additive manner (Neitz et al. 1991; Kawamura et al. 2001; Yokoyama and Radlwimmer 2001; Kawamura and Kubotera 2003; Hiramatsu et al. 2004).

Molecular phylogenies reconstructed from the M/LWS opsin gene sequences show that alleles often cluster by species rather than by allele types and that catarrhine L and M genes form a separate cluster from the New World monkey alleles, implying that alleles were formed in many species independently and that spectral differentiation of L and M opsins in catarrhines occurred after the gene duplication (Shyue et al. 1995; Hunt et al. 1998). However, gene conversion is known to occur frequently between loci and between alleles of M/LWS opsin genes, homogenizing and masking sequence variations among them (Zhou and Li 1996; Boissinot et al. 1998). The spectral differentiation among M/LWS alleles in New World monkeys and prosimians, as well as that between duplicated L and M opsins in catarrhines and howler monkeys, all result from the three-site combinations of the alternative amino acids at each site in common. Thus, it is likely that color vision polymorphism occurred in the common primate ancestor of simians and prosimians. It is also likely that the allelic variation was incorporated and fixed in the same chromosome by a gene duplication in the catarrhine ancestor and independently in howler monkeys (Boissinot et al. 1998; Surridge and Mundy 2002; Surridge et al. 2003; Tan et al. 2005).

The possible antiquity of the M/LWS polymorphism in the primate ancestors, together with the presence of functional SWS1 in many non-lorisiform nocturnal prosimians, implies that trichromacy occurred in the ancestral primates in a polymorphic manner. It is thus likely that the primate ancestor had already shifted to a diurnal or cathemeral pattern from a nocturnal pattern at an early stage. The current nocturnality in many prosimians and in owl monkeys could be a derived state that recurred in several lineages (Tan and Li 1999; Tan et al. 2005).

## 7.4 The Genetic Mechanism of Differential Expression of M/LWS Opsin Subtypes in Primates

Given the spectral differentiation among opsin types and subtypes, a genetic mechanism is required to achieve primate trichromacy that enables differential expression of spectrally distinct opsin genes in different cones. Distinction of expression between SWS1 and M/LWS opsin genes is ancient, dating back to the early vertebrates. The relevant regulatory regions and transcription factors, however, largely remain unsolved. In contrast, the mechanism regulating the expression between M/LWS subtypes in different cones, enabling primate trichromacy, is well understood.

The X-chromosomal locality of the M/LWS type opsin gene in placental mammals is extremely important for the mutually exclusive expression of its subtypes. In females, an allele from only one X chromosome is expressed by virtue of random X-chromosomal inactivation (lyonization), and in males there is simply only one X chromosome. Trichromatic color vision in female New World monkeys is attained by this X-chromosomal locality of the M/LWS opsin gene.

In catarrhines, an additional mechanism is required to selectively express only one gene from the M/LWS gene array on the X chromosome. This expression is achieved through a process of stochastic interaction of a locus control region (LCR), situated upstream of the gene array, with the promoter of only one gene from the array in one cone (Fig. 7.4a) (Wang et al. 1999; Smallwood et al. 2002). The LCR is originally an enhancer element for the single-copy M/LWS opsin gene of mammals (Wang et al. 1992). The gene duplication excluding the LCR from the duplication unit enabled the catarrhines to achieve mutually exclusive expressions of the duplicated M/LWS opsin genes. Hence, primate trichromacy was achieved through the preexisting gene regulatory systems including the X-chromosome lyonization.

In howler monkeys, a gene duplication of the M/LWS opsin has been documented (Jacobs et al. 1996a). Absorption spectra of the resulting L and M opsins were measured indirectly by electroretinogram (ERG) (Jacobs et al. 1996a), which measures gross electric potential of the retina. The estimated absorption spectra of the two photopigments are in good agreement with those of catarrhine L and M, respectively. Mutually exclusive expression of the L and M opsins has been supported by ganglion recording and microspectrophotometry (MSP) (Saito et al. 2004). A recent behavioral experiment also supported the existence of uniform trichromacy in male and female howler monkeys (Araujo et al. 2008). In contrast to catarrhines, however, the howler LCR is duplicated together with the opsin gene (Fig. 7.4b) (Dulai et al. 1999). This finding implies that howler monkeys have evolved extra machinery to attain the mutually exclusive expression of the L and M opsin genes. Studies of this mechanism have not yet been reported. It is also important to study whether trichromatic color vision is really “uniform” in howler monkey populations with no “defective” variants.

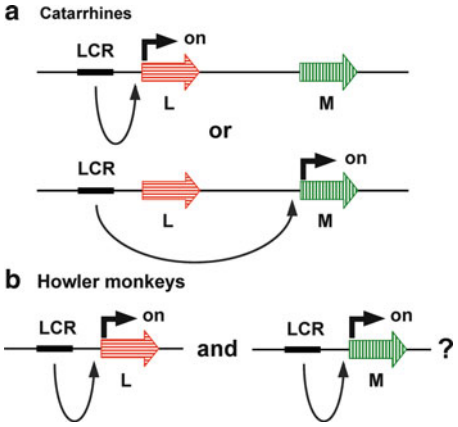


**Fig. 7.4** Mutually exclusive expression of L and M opsin genes. (**a**) In catarrhines, the locus control region (LCR) interacts with either the uppermost (typically L) or the second (typically M) opsin gene in an M/LWS gene array on an X chromosome and turns on the expression of the associated opsin gene. (**b**) In howler monkeys, LCR is duplicated together with the opsin gene and is adjacent to both L and M opsin genes. Without some regulatory mechanism, both L and M opsin genes would be expressed from the same chromosome by the interaction with their LCRs in a given M/LWS cone. The mechanism is currently not known, however

## 7.5 Neuronal Requirement for Evolution of Primate Color Vision

The last mechanistic requirement to achieve primate trichromacy is to establish a neuronal network to convey the color-opponent L–M signals. As in the genetic mechanism for the exclusive gene expression, the necessary neural circuitry was provided serendipitously from a preexisting system for spatial vision.

Most primate features seemingly evolved for a predominantly arboreal life. The visual system of primates, especially that of diurnal simians, is characterized not only by the presence of trichromacy. It is also characterized by forward-facing eyes, the postorbital plate (a bony cup surrounding the eye), and the fovea (a major central peak in cone density in the retina). The forward-facing eyes are seen in all primates and enable stereoscopic vision. The postorbital plate is found in simians and prevents the chewing muscles from disrupting eye position, which could serve to improve visual acuity (Fleagle 1999; Heesy et al. 2007). The simian fovea also allows for very high visual acuity. These features are essential for agile movement and saltatory locomotion from branch to branch.

High spatial acuity is also realized via the evolution of a one-to-one midget ganglion pathway, which receives input from only one M/LWS cone cell in the center of the receptive field of a midget ganglion cell (Martin 1998). This pathway compares the center input to inputs from surrounding M/LWS cones. A more primitive

midget ganglion pathway is seen even in nocturnal prosimians (bushbabies) (Yamada et al. 1998). In bushbabies, the cone–ganglion convergence ratio in the central retina is higher than one (5 cones per ganglion) but is still much lower than that, for example, in cats (~30 cones per ganglion).

The midget ganglion pathway is present only in primates among mammals and conveys information about fine spatial details of the image. When the center-surrounding inputs are from different spectral types of M/LWS cones, the pathway also provides a color opponent mechanism, which is the neural basis of primate trichromacy (Martin 1998).

Recently, knock-in mice that express a human L opsin gene in the form of an X-linked polymorphism have been created (Jacobs et al. 2007). The heterozygous females carry the human L opsin gene and the native mouse M opsin gene. Behavioral tests demonstrated that these mice showed enhanced long-wavelength sensitivity and acquired a new capacity for chromatic discrimination. However, mice lack a midget system. Most retinal ganglion cells in mice have a receptive field center that receives inputs from multiple cone cells. Its antagonistic surround also receives inputs from multiple cone cells. The knock-in mice could extract chromatic information based on the differences in total M versus L input to the two regions. The demonstrated plasticity of the nervous system to accommodate the altered receptor signals is surprising. However, it is questionable whether the new color dimension presented over the coarse spatial image would be useful and adaptive for mice.

Prosimians lack a fovea, and their midget system is relatively unspecialized. Trichromacy by the L–M gene polymorphism is not present in very many species of prosimians. Thus, the selection pressure to maintain trichromacy may not have been strong enough for these mammals with poor spatial resolution of visual images. Without the midget ganglion pathway, evolution of trichromacy would not have been possible in other mammals even if a similar spectral differentiation of opsin subtypes had occurred (Surridge et al. 2003; Vorobyev 2004).

## 7.6 Unsolved Mystery: What Selects for Primate Trichromacy?

### *7.6.1 Fruit Theory*

Although our understanding of the mechanistic aspects of primate trichromacy has greatly advanced in recent years, its adaptive nature is still controversial. It has long been hypothesized that primate trichromacy was selected for finding ripe fruit against a mature leaf background (“fruit theory”) (Allen 1879). In favor of this hypothesis, an analysis of the spectra of fruits eaten by humans showed that the spectral separation of L and M cones is close to optimum for detection of fruit against foliage (Osorio and Vorobyev 1996). This result was later supported by spectral analysis of fruit eaten by primates and collected in the rainforest (Regan et al. 1998, 2001; Sumner and Mollon 2000).

Recently, however, there have been arguments against the fruit theory because many fruits eaten by primates are, in fact, also distinguishable from background leaves by dichromats via the blue–yellow (S vs. L/M) signal and luminance signal (Dominy and Lucas 2001). Furthermore, some fruits do not develop conspicuous colors, yet constitute a significant portion of primate diets, and fruit color seemed to provide no consistent nutritional cue in a study of the 12 plant species most commonly consumed by the primates of Kibale Forest, Uganda (Dominy 2004). Primates are not the sole seed dispersers, and plants also rely on many other frugivores, such as parrots, bats, and peccaries, for successful seed dispersal. In fact, some Old World monkeys are known to often select unripe fruits, which is detrimental for seeds (Dominy and Lucas 2001). In addition, many fruits are highly seasonal and become scarce in dry season.

Figs and palm nuts are not seasonal and can function as keystone resources during the periods of fruit dearth (Terborgh 1986). Cryptic coloration is frequent in figs and palms, and it is suggested that early primates in warm Paleocene-Eocene forests, which were characterized by figs and palms (Morley 2000), relied on them as keystone resources (Dominy et al. 2003b). However, the global cooling and drying during the Eocene–Oligocene interval (~40–30 million years ago) coupled with increasing seasonal fluctuations dramatically reduced densities and availability of figs and palms, especially in Africa (Morley 2000). Africa, where early simians are supposed to have evolved (Fleagle 1999), is still highly seasonal, with a phenology characterized by alternating periods of fruiting and leafing (Dominy et al. 2003b).

### *7.6.2 Young Leaf Theory*

Another theory regarding the evolution of trichromacy is the "young leaf theory." This theory states that, given the seasonality of Africa, young leaves provide a critical fallback resource during periods of fruit shortage (Lucas et al. 1998). Regardless of tree species, young leaves are tender and rich in proteins as well as free amino acids (Dominy and Lucas 2001). Young leaves are often reddish and thus distinct from mature leaves only via the red–green color channel of trichromats. Hence, the ability to discern between young and mature leaves may have been a major selective force for primate trichromacy (Lucas et al. 1998, 2003; Dominy and Lucas 2001). The young leaf theory is strengthened in the context of the historical biogeography of figs and palms; in Africa, where early catarrhines evolved, figs and palms are scarce and routine trichromatic vision was selected for exploiting proteinaceous young leaves as a replacement resource. However, in the Neotropics and Madagascar, where polymorphic color vision is seen in most New World monkeys and some prosimians, figs and palms remained abundant and some New World monkeys, such as marmosets, do not depend on young leaves at all (Dominy et al. 2003b). Thus, the young leaf theory does not seem to explain the evolution and maintenance of trichromacy outside Africa.

### 7.6.3 *Other Theories*

Another longstanding hypothesis to explain the evolution of trichromacy has been the detection of social signals or the detection of predators (Allen 1879; Surridge et al. 2003; Vorobyev 2004; Changizi et al. 2006). A recent study showed, however, that primate trichromacy appeared before the evolution of red pelage and red skin, as well as gregarious mating systems, and therefore the social signals could not be a factor in the evolution of trichromacy from dichromacy (Fernandez and Morris 2007).

Another hypothesis recently put forward could be named the "long-distance foliage hypothesis" (Sumner and Mollon 2000). For trichromatic primates, perceived color, that is, chromaticity, can be described as a ratio of the quantum catch among their L, M, and S cones and expressed as a point in color space analogous to the MacLeod–Boynton diagram (MacLeod and Boynton 1979) consisting of L/(L+M) and S/(L+M) axes. The former axis represents a ratio of quantum catch of L cones to that of L and M cones whereas the latter represents that ratio for S cones to L and M cones (Regan et al. 1998). L/(L+M) indicates the redness that is provided by the "red–green" chromatic channel, for which only trichromats are equipped, and subserved by the midget ganglion cells. The S/(L+M) indicates the blueness that is provided by the "blue–yellow" chromatic channel, for which all mammals are equipped. This more ancient system is subserved by the small bistratified ganglion cells. Colorimetric measurements of natural scenes in forests reveal that the chromaticity of mature leaves falls in a very narrow range of L/(L+M) values but spreads widely along the S/(L+M) axis and also in luminance values. Thus, the chromaticity of fruits, young leaves, pelage, and skin often deviates from mature leaves in L/(L+M) value but largely overlaps with them in S/(L+M) and luminance values (Regan et al. 1998, 2001; Sumner and Mollon 2000, 2003), leading to a hypothesis that primate trichromacy could be adaptive for and have evolved for detecting *anything* differing from the background foliage in L/(L+M) value. This trichromat advantage is supposed to be maximized during long-distance viewing because the scene would contain a larger variety of background S/(L+M) and luminance values than would a closer view. In addition, during close viewing, other sensory cues, such as odors, are available and visual cues could be less important (see also Chap. 5).

On the other hand, the "short-distance foliage hypothesis" is also suggested by psychophysical studies. The human visual system shows a relatively greater sensitivity to low spatial frequencies of chromatic spatial modulation than to luminance spatial modulation (Mullen 1985). In addition, a statistical analysis of spatial frequencies of natural images suggests that the spatiochromatic properties of the red–green system of human color vision may be optimized for the encoding of any reddish or yellowish objects against a background of foliage at relatively small viewing distances commensurate with a typical grasping distance (Parraga et al. 2002).

### 7.6.4 *Advantage of Dichromacy*

Recent studies have found that dichromatic vision may be advantageous to primates under some conditions, for example, finding cryptic fruits or insects or detecting cryptic predators, such as snakes (Caine et al. 2003; Saito et al. 2005b). The conceptual basis for this hypothesis is that trichromatic vision compromises the acuity of other visual systems. The neural system of trichromatic individuals must combine signals from the L and M photoreceptors to obtain the luminance signal used for achromatic "colorblind" tasks such as spatial vision and the perception of shape, texture, and motion (Morgan et al. 1992; Kelber et al. 2003). The different spectral inputs from the two receptors can cause corruption, resulting in a weaker overall signal. Additionally, color may compete with texture information, or trichromats may learn to rely on color at the expense of information to be gained by texture. Therefore, dichromats may have an advantage over trichromats in achromatic (colorblind) tasks, such as depth perception and breaking camouflage.

### 7.6.5 *Behavioral Experiments*

Behavioral experiments that compared feeding efficiency between vision types in laboratory settings with artificial targets have suggested that there is a selective advantage of trichromacy in foraging on colored foods (Caine and Mundy 2000; Caine 2002; Smith et al. 2003b). On the other hand, these studies and others have found that dichromats are better than trichromatic primates at detecting camouflaged stimuli (Caine et al. 2003; Saito et al. 2005b). It should be noted that such comparisons evaluate whether the difference in visual ability is consistent with the difference in color vision phenotypes but do not evaluate whether one phenotype is more advantageous than another (Saito et al. 2005a). These experiments should only be regarded as tests that determine visual phenotypes, although they do provide useful predictions about the potential foraging advantages of dichromatic and trichromatic phenotypes.

## 7.7 New World Monkeys as a Model to Study the Adaptive Nature of Primate Color Vision

Whatever the theories and laboratory experiments predict, the adaptive value of primate trichromacy (or dichromacy) can only be evaluated in light of behaviors seen in natural environments. Hence, it is important to compare behaviors between free-ranging dichromats and trichromats and to evaluate whether and how the contrast between a visual target and its background are correlated with behavioral differences in these two types of primates. New World monkeys are an excellent

model to test the suggested advantage of trichromacy because of the allelic polymorphism of the L–M opsin gene that results in coexistence of dichromatic and trichromatic individuals in the same population (Mollon et al. 1984) (Fig. 7.2). Prerequisites for realizing these studies are that a method must be established to determine vision phenotypes without harming the study animals and that the monkeys at the study site must be well habituated and individually identified by researchers. In the next two subsections, we review the methodology related to the first prerequisite and introduce our study site as an example. Then, we summarize recent findings, including ours, on vision–behavior relationships in wild New World monkeys. We also introduce our population genetic study on natural selection acting to maintain color vision variation in the monkeys at our study site.

### *7.7.1 Spectral Genotyping of L–M Opsin Alleles for Field Samples*

The spectral genotype of the L–M opsin gene can now be estimated noninvasively through polymerase chain reaction (PCR) and DNA sequencing analyses of fecal samples collected in the field (Surridge et al. 2002; Hiramatsu et al. 2005). This estimation can be done by examining exon 3 (containing the residue 180) and exon 5 (containing the residues 277 and 285) of the L–M opsin gene (Hiramatsu et al. 2004) (see Sect. 7.3.3). The estimated absorption spectra can be confirmed experimentally by reconstitution of the opsin photopigments in vitro and direct measurement of their absorption spectra (Yokoyama 2000b). With a single S opsin gene, female monkeys having two different spectral alleles of the L–M opsin gene are considered trichromats, and females having two identical L–M opsin alleles, and males, because of the hemizygosity of the X chromosome, are considered dichromats. Consistency between the genotype and phenotype has been well demonstrated by behavioral experiments (Caine and Mundy 2000; Saito et al. 2005a).

### *7.7.2 An Example Study*

To study vision–behavior relationships in wild monkeys, it is essential that monkeys in the study site be habituated to human observers and individually identified. It is also important that ecological and sociological information be available, and it is desirable that two or more primate species with different ecology, phylogeny, and vision types be present at the site for comparative purposes. We have chosen the Santa Rosa Sector of the Área de Conservación Guanacaste (ACG), northwestern Costa Rica (10°45′ to 11°00′ N and 85°30′ to 85°45′ W), as an ideal study site where prerequisite conditions are satisfied (Chapman 1990; Fedigan and Jack 2001, 2004; Fedigan 2003). The park is composed of tropical dry forest in various successional

stages. Rainfall in Guanacaste Province is highly seasonal; mean annual rainfall is approximately 2 m, almost all of which is accumulated between mid-May and mid-December (Janzen 2002).

There are three sympatric primate species at Santa Rosa: white-faced capuchin monkeys (*Cebus capucinus*), black-handed spider monkeys (*Ateles geoffroyi*), and mantled howler monkeys (*Alouatta palliata*). The first two genera of monkeys are known to have color vision polymorphism (Jacobs 1998). Spider and capuchin monkeys differ in diet (frugivores and omnivores, respectively), social structure (male philopatry with high fission–fusion dynamics and female philopatry with rather cohesive groups, respectively) (Fragaszy et al. 2004; Aureli and Schaffner 2008), and phylogeny (Atelidae and Cebidae, respectively) (Schneider 2000). Capuchins are reported to possess three L–M opsin alleles, whereas spider monkeys possess two (Shyue et al. 1998; Jacobs and Deegan 2001). These conditions make capuchins and spider monkeys excellent study subjects, complementary to callitrichine species (marmosets and tamarins), whose vision–behavior relationships are the most intensively investigated among New World monkeys (Caine and Mundy 2000; Caine 2002; Caine et al. 2003; Pessoa et al. 2003; Smith et al. 2003a, b).

On the basis of the three-site rule, we identified three L–M opsin alleles in capuchin monkeys, one having the three-site composition Ser, Tyr, and Thr at sites 180, 277, and 285, respectively (designated Ser/Tyr/Thr), and the other two having Ala/Phe/Thr and Ala/Phe/Ala (Hiramatsu et al. 2005). The $\lambda_{max}$ values of the three alleles were directly measured by the method of photopigment reconstitution in vitro and were determined to be 561, 543, and 532 nm (designated P561, P543, and P532), respectively (Hiramatsu et al. 2005). Similarly, we identified two L–M opsin alleles in spider monkeys, one having the three-site composition Ser/Tyr/Thr and the other having Ser/Phe/Thr (Hiramatsu et al. 2005). The $\lambda_{max}$ values of the two alleles were directly measured in vitro and were determined to be 553 and 538 nm, respectively (designated P553 and P538) (Hiramatsu et al. 2008). In a spider monkey group, the two alleles, P553 and P538, are present at 59.6% and 40.4%, respectively, among 52 X chromosomes examined (20 females and 12 males) (Hiwatashi et al. 2010). In a capuchin monkey group, the three alleles, P561, P543 and P532, are present at 51.6%, 35.5% and 12.9%, respectively, among 31 X chromosomes examined (8 females and 15 males) (Hiwatashi et al. 2010). In both species, the genotypes are present at frequencies that do not deviate significantly from Hardy–Weinberg equilibrium (Hiwatashi et al. 2010). These results suggest that there is little effect of color vision phenotype on mate choice, or with the small sample sizes here, effect of color vision phenotype on mate choice, if any, is not strong.

### *7.7.3 Behavioral Observations on Wild Populations of New World Monkeys*

In recent years, many studies, including ours, have been published on behavioral observations of wild populations of New World monkeys for evaluating vision–behavior relationships. Despite the predicted advantage of trichromacy, these

studies have provided only limited support. In a study of a wild mixed-species troop of saddleback (*Saguinus fuscicollis*) and mustached (*S. mystax*) tamarins, during vigilance, trichromats are further from their neighbors than their dichromatic conspecifics, which is explained as resulting from the potentially better perception of predation risk in trichromats (Smith et al. 2005). Our study of a population of white-faced capuchin monkeys (*C. capucinus*) found that dichromats sniffed more figs and had longer foraging sequences than trichromats, especially for cryptic figs (Melin et al. 2009). Among six subtypes of dichromats and trichromats, monkeys possessing the trichromat phenotype with the most spectrally separated L–M opsin alleles showed the highest acceptance index for conspicuous figs, although there were no differences in feeding rates among phenotypes (Melin et al. 2009).

Results of other behavioral observations of wild New World monkeys have produced equivocal results or results contradictory to the predictions from the trichromat advantage hypothesis. The study of the wild mixed-species troops of tamarins showed that the color vision types (dichromatic or trichromatic) did not have a consistent effect on the leadership of the troops to feeding trees (Smith et al. 2003a). Another study of tamarins (*S. imperator imperator* and *S. fuscicollis weddelli*) found no significant difference between females (thought to consist of trichromats and dichromats) and males (all dichromats) in their ability to locate or discriminate between feeding sites (Dominy et al. 2003a). In a population of capuchin monkeys (*C. capucinus*), there was no significant difference between trichromats and dichromats in feeding or energy intake rates (Vogel et al. 2007). In another population of the same capuchin monkey species, we showed that there was no difference between dichromats and trichromats in time spent foraging on different food types (Melin et al. 2008).

Some modeling studies based on field observations have found that many fruits eaten by spider monkeys or squirrel monkeys (*Saimiri sciureus*) are similarly discernible or similarly indiscernible from background foliage for both trichromats and dichromats (Riba-Hernández et al. 2004; Stoner et al. 2005; De Araujo et al. 2006). Our field study of free-ranging black-handed spider monkeys measuring their foraging efficiency on fruits and colorimetric properties of fruits and background leaves revealed that dichromats are not inferior to trichromats in frequency, accuracy, and unit-time intake efficiency of detecting fruits (Hiramatsu et al. 2008). We showed that this is because the luminance contrast of fruits to background leaves is the main determinant of fruit detection in both dichromats and trichromats. Another study of ours on the same social group of spider monkeys also showed that, irrespective of color vision phenotypes, the monkeys sniff visually cryptic fruits more often than visually conspicuous fruits (Hiramatsu et al. 2009). This finding indicates that color vision is not the sole determinant for ingestion or rejection of fruits. Our field study of white-faced capuchin monkeys has even demonstrated a dichromat advantage in foraging for surface-dwelling insects (Melin et al. 2007, 2010). These findings of observational studies in natural environments suggest that the superior ability of trichromats to see the red–green color contrast may not translate into a selective advantage because the use of a variety of sensory modalities may compensate for the inferiority of any one sense (Hiramatsu et al. 2009) (see also Chaps. 4–6).

### 7.7.4 Population Genetic Analysis of L–M Opsin Gene Polymorphism in the Wild Populations

As we have already summarized, field observations of the foraging behaviors of New World monkeys have thus far mostly failed to detect a clear advantage of trichromacy or have even demonstrated a dichromat advantage for insect foraging. This situation leaves a fundamental question unanswered regarding what maintains trichromatic vision in New World monkeys, because trichromacy (i.e., heterozygosity on the L–M opsin alleles) would have disappeared without a selective force acting to maintain allelic variations of the L–M opsin.

Interspecies comparisons of the L–M opsin gene sequences among primates and others have found signatures of positive natural selection for generating trichromatic color vision and have identified relevant amino acid substitutions for the selection effective in spectral differentiation between the L and M opsin genes (Yokoyama and Yokoyama 1990; Neitz et al. 1991; Shyue et al. 1995, 1998; Boissinot et al. 1998). The color vision polymorphism is transspecific and is documented in all three families of the New World monkeys (Atelidae, Pitheciidae, and Cebidae) (Jacobs 2007). The long duration of the polymorphism in these Neotropical primate families is consistent with balancing selection, a form of positive natural selection, operating to maintain variation via heterozygote advantage of trichromatic females (Boissinot et al. 1998; Surridge and Mundy 2002; Surridge et al. 2003).

However, the effective population size $N_e$, a major determinant of the duration of allelic turnover, remains unknown for New World monkeys. Assuming that the last common ancestor of all New World monkeys originated 26 million years ago (Schneider 2000), it follows that the opsin polymorphism has persisted over this period. In theory, the expected survival time for a neutral X-linked allele is $3N_e$ generations in a stationary population. If $N_e$ of New World monkeys had been large enough (e.g., of the order of $10^6$) in the long-isolated South American continent without formidable eutherian predators, then the polymorphism could have persisted for this length of time. Another problem is that it is difficult to estimate $N_e$ in a natural population. The estimated value of $N_e$ depends on the accuracy of estimates of the mutation rate and generation time and is confounded by demographic effects such as the historical dynamism of population size, migration pattern, and population structure. Although demographic effects influence genetic variation of all genes in the genome alike, the pattern and the intensity of natural selection can vary among genomic regions depending on direct or indirect effects of mutations in that region to fitness.

It is thus necessary to apply a method that compares the pattern of intraspecific genetic variation between a focal region (i.e., the L–M opsin gene) and other reference regions in the same genome using the same population samples to cancel out the effects of demographic factors which both regions share (Verrelli and Tishkoff 2004; Perry et al. 2007; Verrelli et al. 2008). We employed this approach for a group of spider monkeys and a group of capuchin monkeys from the Santa Rosa populations described in Sect. 7.7.2 (Hiwatashi et al. 2010).

Hiwatashi et al. (2010) evaluated the three basic parameters, $\pi$, $\theta_w$, and Tajima's *D*, to describe the level of nucleotide variation within a population. Nucleotide diversity ($\pi$) is the average number of nucleotide differences per nucleotide site between two sequences (and is also the unbiased estimator of the average heterozygosity among nucleotide sites) (Nei and Kumar 2000). The number of polymorphic (segregating) sites among samples (*S*) derives a nucleotide polymorphism parameter $\theta_w \equiv S / L / \sum_{i=1}^{n-1} \frac{1}{i}$, where *L* is the length of the sequence and *n* is the number of samples (Watterson 1975). In theory, when mutations are selectively neutral and population size is constant through generations, both $\pi$ and $\theta_w$ are expected to converge to the population mutation rate $\theta$ ($\equiv 4N_e\mu$ for autosomal and $3N_e\mu$ for X-chromosomal genes of diploid organisms, where $N_e$ is the effective population size and $\mu$ is the mutation rate per nucleotide site per chromosome per generation). Tajima's *D* evaluates the difference between $\pi$ and $\theta_w$, which is given by $\pi - \theta_w$ divided by the estimated standard error of the difference (Tajima 1989).

The Tajima's *D* value of neutral references can be regarded as a control measure of severity of demographic effects. If Tajima's *D* of neutral references is positive, it could imply a long-term reduction of the population size or recent admixture of genetically differentiated subpopulations. If it is negative, it could imply a long-term expansion of population size or recent incorporation of genetically differentiated minority. On top of this, if the Tajima's *D* value of the L–M opsin gene is positive and significantly larger than that of neutral references, this is taken as an evidence of balancing selection operating on the L–M opsin gene. We used a computer simulation ("coalescence simulation") to determine whether the observed values of the three parameters are deviated with statistical significance from expectation under the assumption of neutrality and constant demography.

We also evaluated nucleotide divergence between spider monkey and capuchin monkey populations. The nucleotide divergence between species is defined as the proportion of nucleotide sites where one species is monomorphic (i.e., fixed) with a nucleotide and the other species is fixed with another nucleotide.

We showed that the nucleotide sequence of the L–M opsin gene was significantly more polymorphic than the sequences of the neutral references in terms of $\pi$ and $\theta_w$ in both spider monkeys and capuchin monkeys. The Tajima's *D* value of the L–M opsin gene also deviated significantly in a positive direction from the neutral expectation in both species. In particular, this deviation from neutrality was evident in the central part of the L–M opsin gene region, including exon 3 and exon 5, which encode the spectrally important amino acid sites. On the other hand, viewed from nucleotide divergence between the two species, L–M opsin gene sequences were not more divergent than the sequences of the neutral references. Within the L–M opsin gene region, the central region was not more divergent between species than the peripheral region. The nucleotide divergence data confirm that the larger within-species variation in L–M opsin gene than neutral references, especially in its central region, is not the result of a difference of mutation rate among these regions.

In addition to the results shown in Hiwatashi et al. (2010), we evaluated whether the ratio of polymorphic to fixed sites was different between genomic regions by

**Table 7.1** The number of polymorphic and fixed nucleotide sites in the neutral references and the L–M (red–green) opsin gene regions in spider and capuchin monkeys

| Region | Length (bp) | No. of polymorphic sites (%) | No. of fixed sites (%) |
|---|---|---|---|
| Neutral reference | 2,045 | 20 (1.0) | 103 (5.0) |
| L–M opsin | | | |
| Exon 1 | 881 | 7 (0.8) | 35 (4.0) |
| Exon 3 | 949 | 42 (4.4) | 26 (2.7) |
| Exon 5 | 827 | 34 (4.1) | 13 (1.6) |
| Exon 6 | 835 | 23 (2.8) | 39 (4.7) |
| Total | 3,492 | 106 (3.0) | 113 (3.2) |

*Note:* The set of sequences used is the same as in Table 7 of Hiwatashi et al. (2010)

the conventional $\chi^2$ test (a simplified HKA test) (Hudson et al. 1987) (Table 7.1). The ratio of polymorphic to fixed sites was significantly higher in the L–M opsin gene region than in the neutral references ($\chi^2 = 35.0$, $df = 1$, $P < 0.0001$). Within the L–M opsin gene, the ratio is also significantly higher in the central region (exons 3 + 5) than in the peripheral region (exons 1 + 6) ($\chi^2 = 30.3$, $df = 1$, $P < 0.0001$). All these results are explained only by the action of balancing selection to the spectrally important amino acid sites located in the central region of the L–M opsin gene. The study by Hiwatashi et al. (2010) is the first to statistically demonstrate balancing selection acting on the polymorphic L–M opsin gene of New World monkeys.

### *7.7.5 What Is the Nature of Balancing Selection on the L–M Opsin Gene?*

Given the clear indication of balancing selection obtained in our study and the uncertainty about benefits of trichromacy (i.e., for individuals heterozygous for the L–M opsin alleles) resulting from behavioral studies of wild primates (Dominy et al. 2003a; Smith et al. 2003a; Melin et al. 2007; Vogel et al. 2007; Hiramatsu et al. 2008, 2009), how should we interpret the nature of balancing selection? Several advantages of trichromacy have been proposed, such as long-distance detection of reddish objects under dappled foliage (Sumner and Mollon 2000), foraging on reddish ripe fruits in severe dry seasons when these could be scarce (Dominy and Lucas 2001), and recognition of social signals and predators (Changizi et al. 2006; Fernandez and Morris 2007). Critical behavioral data to demonstrate these advantages have yet to be gathered, however.

Besides the trichromat advantage, three other mechanisms of balancing selection have been hypothesized to explain color vision polymorphism in New World monkeys (Mollon et al. 1984): (1) negative frequency-dependent selection, which predicts the fitness of any given phenotype to be reciprocal to the frequency of that phenotype in the population; (2) niche divergence, which predicts that individuals of each phenotype will specialize in a distinct visual or ecological niche or visual ability; and

(3) mutual benefit of association, which predicts that individuals of each phenotype benefit from being associated with individuals of other phenotypes in a polymorphic group.

Negative frequency-dependent selection is generally invoked for predator–prey interaction or a disassortative mating system, that is, mating with a different type from oneself in terms of the genetic trait in question (Conner and Hartl 2004). This case is hard to envision in the case of color vision and often appears to be interpreted mistakenly in literatures as a consequence of niche divergence (thus the two hypotheses are often confounded). Under the niche-divergence situation the population size of each phenotype can fluctuate independently from each other and irrespectively of its frequency because individuals with different phenotypes exploit different resources or niches and population size of the phenotype changes as the carrying capacity of their niche changes but not as population size of another phenotype changes. Although negative frequency-dependent selection is often referred as an alternative explanation to the heterozygote advantage hypothesis as a mechanism for maintaining color vision polymorphism, it would be the least likely mechanism.

Few studies evaluate the niche divergence hypothesis. In the food foraging behaviors of capuchin monkeys, there is no difference in foraging time spent on different food types between dichromatic and trichromatic individuals (Melin et al. 2008). However, more studies are needed to test this hypothesis.

Although no study has evaluated the mutual benefit hypothesis, balancing selection may represent an advantage of individuals associated with different color vision phenotypes coexisting in the same population. There is a clear advantage of monkey and ape dichromats, as well as human dichromats, in detecting color-camouflaged objects (Morgan et al. 1992; Caine et al. 2003; Saito et al. 2005b), including surface-dwelling insects (Melin et al. 2007, 2010), an important food source for many primates. Humans are polymorphic in color vision and have a long history of a hunting and gathering lifestyle in which the ability to break camouflage may be advantageous. We thus need to ask whether the selective advantages of dichromacy are applicable to evolution of human color vision. Given such potential selective advantages in dichromats, we also need to ask why dichromats are so rare in nonhuman catarrhines.

## 7.8 Conclusions

Color vision of primates is unique among vertebrates in its evolutionary history. Trichromatic color vision in primates was generated from dichromatic color vision seen in other mammals by allelic differentiation or gene duplication of the M/LWS type opsin gene that had evolved in an early vertebrate ancestor. The necessary mechanism for mutually exclusive expression of the L and M opsin gene in a cone cell was provided by the X-chromosomal locality of the M/LWS type opsin gene in mammals and a preexisting enhancer element for it. The necessary mechanism for neuronal processing was also provided by a preexisting system for acute spatial

vision. We showed that the polymorphic color vision of New World monkeys is maintained by natural selection. But there is still controversy over the advantages of trichromatic color vision and of polymorphic color vision. A deeper knowledge of primate color vision will facilitate our understanding of human color vision. Nonhuman primates are a good reference point for comparison. Studies of New World monkeys are particularly important for understanding a condition where color vision can be polymorphic in the population. Since the isolation of cone opsin genes in the mid-1980s (Nathans et al. 1986), our understanding of the evolution of color vision has progressed rapidly, largely because these studies encompass research on genes, physiology, and behavior. Further interdisciplinary studies will provide a wealth of data for increasing our understanding of the evolution of color vision.

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