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Origin and Molecular Evolution of the X-linked Duplicate Color Vision Genes in Howler Monkeys

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Stephane Boissinot, Yi-Hong Zhou, Li Qiu, Kanwaljit S. Dulai, Katherine Neiswanger, Horacio Schneider, Iracilda Sampaio, David M. Hunt, David Hewett-Emmett and Wen-Hsiung Li (1997) Origin and molecular evolution of the X-linked duplicate color vision genes in howler monkeys. *Zoological Studies* **36**(4): 360-369. Like humans and Old World monkeys (OWMs), the howler monkeys, a genus of New World monkeys (NWMs), have trichromatic vision because they possess 1 autosomal (blue pigment) and 2 X-linked (red and green pigments) color vision genes. In contrast, the other NWM species investigated in detail have only 1 autosomal and 1 X-linked color vision gene, though the X-linked locus is polymorphic with 3 alleles. To understand the origin of trichromacy in howler monkeys, several NWM species were examined for the number of X-linked pigment loci, and intron 4, and exons 3, 4, and 5 of the red and green pigment genes of a male howler monkey were sequenced. The spider monkey, the woolly monkey, the saki monkey, and the bearded saki monkey were shown by the technique of single strand conformational polymorphism (SSCP) and by Southern blotting to have only 1 X-linked color vision gene, suggesting that within NWMs, the howler monkeys are the only genus with 2 X-linked pigment loci. The sequences of exons 3, 4, and 5 and intron 4 reveal that the gene duplication in the howler monkey was independent of that in the human-ape-OWM lineage. In addition, the amino acids at 4 critical sites for spectral tuning suggest that the duplication in the common ancestor of howler monkeys was derived from the incorporation of 2 alleles that were, respectively, very similar to the P535 (green) and P562 (red) pigment alleles currently existing in the squirrel monkey and capuchin (2 NWM genera). This hypothesis implies that the P535-P562 polymorphism existed before the platyrrhini (NWM) radiation, which took place about 20 million years ago. Furthermore, the distribution of sequence differences in intron 4 between the 2 howler monkey genes suggests that the 2 intron 4 sequences have been homogenized by recent gene conversion events, providing further evidence for the frequent occurrence of gene conversion between X-linked pigment genes.

Key words: Trichromacy, Color pigment, New World monkeys, Ancient polymorphism.

Humans, apes, and Old World monkeys (OWMs) are trichromatic because they possess 1 autosomal gene that encodes a blue-sensitive pigment and at least 2 X-linked genes that encode the red- and green-sensitive pigments (Nathans et al. 1986, Ibbotson et al. 1992, Neitz and Neitz 1995). The latter 2 genes are believed to have arisen from a gene duplication before the separation of the ape-human lineage from the OWM lineage, but after the separation of the OWM-ape-human lineage from the New World monkeys (NWMs). On the other hand, most species of NWMs have only 1 X-linked and 1 autosomal color photopigment gene. However, detailed studies in squirrel monkeys, marmosets, and related species reveal that the X-linked locus has 3 alleles coding for 3 phenotypically different pigments

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(Jacobs 1984, Mollon et al. 1984, Jacobs and Neitz 1987a, Travis et al. 1988, Williams et al. 1992, Jacobs et al. 1993). Because of this triallelic system at the X-linked photopigment locus, heterozygous female monkeys are trichromatic, though males and homozygous females are dichromatic.

It has been believed that all NWMs have only 1 X-linked photopigment gene. However, it has recently been demonstrated that, like humans, apes, and OWMs, the howler monkey (a NWM) has 2 X-linked genes that encode 2 pigments with spectral sensitivity maxima near 535 and 560 nm, respectively (Jacobs et al. 1996). The 2 pigments will be called the "green" and "red" pigments because their spectral peaks are close to those of the human green and red visual pigments. The 2 X-linked pigment genes in howler monkeys could have arisen from a relatively recent duplication, that is independent of the duplication for the 2 X-linked pigment genes in the human-ape-OWM lineage (i.e., the independent origin hypothesis). Alternatively, the duplication in the howler monkey could have had the same origin as that in the common ancestor of humans, apes, and OWMs (i.e., the single origin hypothesis). It has been proposed that the former hypothesis is more likely (Jacobs et al. 1996), but it is interesting to determine if the hypothesis is supported by further data and whether the duplication exists only in the howler monkeys.

To study the origin and evolution of the 2 X-linked pigment genes in howler monkeys, we decided to examine the number of X-linked pigment genes in other NWMs. We also decided to sequence exons 3, 4, and 5 and intron 4 of the 2 genes in a male howler monkey. These 3 exons are interesting because they contain 4 critical positions at which amino acid differences can largely account for the differences in spectral sensitivity among visual pigments in higher primates (Neitz et al. 1991, Merbs and Nathans 1993, Shyue et al. 1997). These positions can provide useful phylogenetic information because noncritical regions, like intron 4, can be homogenized by gene conversion (Shyue et al. 1994, Zhou and Li 1996, Boissinot et al., unpubl. data). From this point of view, we were also curious to know if gene conversions have also occurred between introns 4 of the 2 duplicated genes in the howler monkey.

MATERIALS AND METHODS

DNA sample sources

Two males and 1 female howler monkey (*Alouatta seniculus*) were used in this study; 1 male is of unknown origin and the other male and the female were from natural populations in French Guyana. Other primates used in this study are two species of spider monkey (1 male *Ateles geoffroyi* of unknown origin, and two male *Ateles chamek* monkeys from Brazil), 1 male woolly monkey (*Lagothrix lagothricha*, unknown origin), 3 male saki monkeys (*Pithecia pithecia*, 1 from Brazil and 2 from French Guyana), 2 male bearded saki monkeys (*Chiropotes albinasus*, Brazil), and 1 female and two male tamarins (*Saguinus mystax*, Peru). All DNAs were extracted from blood samples.

SSCP analysis

SSCP (single strand conformation polymorphism) (Mundy 1997) was performed to detect the existence of multiple copies of the X-linked pigment gene in an individual. Exon 3, which contains 1 critical site, and exon 5, which contains 2 critical sites, were chosen for the SSCP analysis. Exon 3 was amplified by the polymerase chain reaction (PCR) using primers 5'-ATCACAGGTCTCTGGTCTCTGG-3' (sense) and 5'-ACCTGCTCCAACCAAAGATGGG-3' (antisense). Exon 5 was amplified using primers 5'-TGGCAAAGCAGCAGAAAGAGTC-3' (sense) and 5'-CTGCCGGTTCATAAAGACATAG-3' (antisense). PCR amplifications were carried out in 50-µl reaction mixtures containing 10 mM Tris-HCl (pH 9.0), 50 mM KCl, 0.1% Triton X-100, 1.5 mM $MgCl_2$, 120 µM dNTP, 1 unit of Taq DNA polymerase (Promega), 50 ng of each primer, and 0.1 µl of (α-^{32}P)dCTP (Amersham). Exons 3 and 5 were amplified from 50 ng of genomic DNA with 30 cycles of 94 °C (1 min), 60 °C (1 min), and 72 °C (1.5 min). Six microliters of formamide dye were added to the PCR mixture. The mixture was denatured at 95 °C (3-5 min) and 6 µl was loaded on an MDE gel, 0.6x TBE. The gel was electrophoresed overnight (14 h) at 6 W and room temperature.

SSCP is a technique that allows detection of differences between sequences. This method has been demonstrated to be effective in detecting polymorphisms because it is sufficiently sensitive to detect single base differences. The way we used this technique is based on the same principle. We assume that trichromacy in primates results from the presence on the X chromsome of 2 (or more) copies of the pigment gene which differ at critical sites. By amplifying the portion of the sequence where critical sites are located (like in exons 3 and 5), we can detect the number of copies that differ in sequence in a given male individual. Of course, if multiple copies

are present in an individual but do not differ at any sites, we will not be able to detect the existence of multiple copies. This is not a problem because we are interested in the number of functionally different genes.

Southern blot analysis

Ten micrograms of genomic DNA were digested with 20 units of *Eco*RV restriction enzyme and separated by overnight electrophoresis through 0.7% agarose gels at 35 mV, then DNA was transferred to a nylon membrane (Hybond N^+, Amersham) according to the Southern method. The DNA was immobilized by UV exposure for 3 min. Membranes were prehybridized at 68 °C for 2 h in hybridization buffer (6x SSC, 0.1% SDS, 0.02% Ficoll). The probes were generated by PCR using the same 2 primers for amplifying exon 5 in the SSCP analysis, and labeled by random priming with (α-^{32}P)dCTP. Hybridization was conducted overnight at 68 °C in 3x SSC, 0.1% SDS, 0.02% Ficoll. The membranes were washed 3 times at 68 °C with 2x SSC/0.1% SDS.

PCR amplification, cloning, and sequencing

Exons 3, 4, and 5, and intron 4 were amplified by PCR. Exon 3 was amplified using primers 5'-GCAGAATGGCATGCGGAAACAAACTG-3' (sense) and 5'-CCAGAGAAAGGAAGTGATTTGCC-3' (antisense). A region covering exon 4, intron 4, and exon 5 was amplified using 3 pairs of primers that amplified overlapping fragments. The segment covering exon 4 and the 5' end of intron 4 was amplified using primers 5'-GCCGGCCCTTCTCTCCAG-3' (sense) and 5'-CCCAGTGGCAACCTAGTCTAGG-3' (antisense); the segment covering the 3' end of intron 4 and the 5' end of exon 5 was amplified using primers 5'-CCAGCGAGAAGAATCAGGTGATTCC-3' (sense) and 5'-ACCACCAGCATGCGCGTCACTTC-3' (antisense); and the entire exon 5 was amplified using primers 5'-ATCCTCCAATTCTAGAAGGACTCCC-3' (sense) and 5'-TCAGAGACATGATTCCAGGTGG-3' (antisense). PCR amplifications were carried out in 60-µl reaction mixtures containing 10 mM Tris-HCl (pH 9.0), 50 mM KCl, 0.1% Triton X-100, 1.5 mM $MgCl_2$, 120 µM dNTP, 1 unit of Taq DNA polymerase (Promega), and 50 ng of each primer. The sequences were amplified from 200 ng of genomic DNA with 30 cycles of 94 °C (1 min), 60 °C (1 min), and 72 °C (1.5 min).

The amplified DNA fragments were purified using the Magic PCR Preps kit (Promega), and cloned into *Eco*RV-digested p-Bluescript SK^+ TA cloning vector. The ligations were then used to transform competent *Escherichia coli* XL1-Blue cells.

Plasmid DNA for sequencing was purified with the Wizard Minipreps kit (Promega). For each ligation, several clones were sequenced. We first sequenced exon 5, which was known to contain 2 of the 4 critical sites (positions 277 and 285). Using these positions, and by comparing them with other species, we were able to identify 2 types of sequence. For each type of sequence, 5 clones and both strands of DNA were sequenced. Internal sequencing primers were designed as sequence information accumulated.

Data analysis

Sequences were aligned manually. The MEGA program package (Kumar et al. 1993) was used for phylogenetic reconstruction. Kimura's 2-parameter distances (Kimura 1980) were computed and used to build phylogenetic trees by the neighbor joining method (Saitou and Nei 1987).

RESULTS AND DISCUSSION

SSCP and Southern blot analysis

The SSCP analysis gave 2 different patterns: in some individuals a single band was observed, whereas in the other individuals 2 bands were observed (Fig. 1). For a given individual, the amplification of exons 3 and 5 gave the same number of bands. A single band was obtained in males of the following species: *Ateles geoffroyi, Ateles chamek, Lagothrix lagothricha, Pithecia pithecia, Chiropotes albinasus*, and *Saguinus mystax*. In contrast, 2

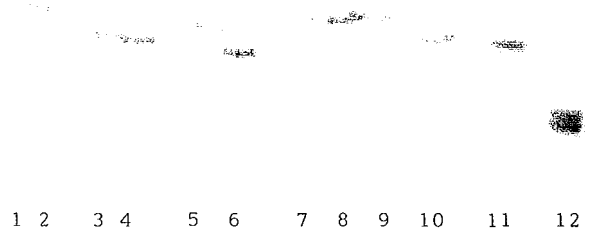

Fig. 1. SSCP for exon 5. Lane 1, male howler monkey; Lane 2, female howler monkey; Lanes 3 and 4, male saki monkeys; Lanes 5 and 6, male bearded saki monkeys; Lane 7, male spider monkey (*Ateles geoffroyi*); Lanes 8 and 9, male spider monkey (*Ateles chamek*); Lane 10, wolly monkey; Lane 11, male tamarin; and Lane 12, female tamarin.

bands were obtained in male howler monkeys and in the female tamarin (*Saguinus mystax*). The presence of 2 bands reveal that 2 sequences differing by at least 1 mutation have been amplified simultaneously. In the case of the female tamarin, which is known to have only 1 X-linked pigment gene, the 2 bands indicate that this female is heterozygous at this locus; we verified this result by direct sequencing of exon 5. In the case of the male howler monkey, which has only 1 X chromosome, the 2 bands confirm the presence of 2 X-linked pigment loci and indicate that both exons 3 and 5 differ between the 2 genes. In other species, no evidence of multiple copies was found with this method. We decided to confirm the SSCP results by a Southern blot analysis in 2 close relatives of the howler monkey: the spider monkey *Ateles geoffroyi* and the woolly monkey *Lagothrix lagothricha*. In both species, only 1 band was obtained, supporting the SSCP results.

These results indicate that the 2 species of spider monkey (*A. geoffroyi* and *A. chamek*), the woolly monkey (*L. lagothricha*), the saki monkey (*P. pithecia*), and the bearded saki monkey (*C. albinasus*), like the other NWM species studied earlier, have only a single X-linked photopigment gene. Previous results suggested that, as in the squirrel monkey, a polymorphism exist within spider monkey populations (Blakeslee and Jacobs 1982, Jacobs and Deegan 1993). The dichromacy of each of the males they studied is consistent with the idea that spider monkeys have a single X-linked photopigment gene. Our SSCP analysis clearly confirms this interpretation. In the family Pithecidae (sensus Harada et al. 1995), which includes 4 genera (*Callicebus; Pithecia; Chiropotes; Cacajao*), the previous data suggested the presence of 1 X-linked pigment gene in the titi monkey, *Callicebus moloch* (Jacobs and Neitz 1987b). Here, we report the same situation in *Pithecia* and in *Chiropotes*.

These new results and those of Jacobs et al. (1996) are consolidated in Figure 2. In this figure, the 2 families Atelidae and Pithecidae (sensus Harada et al. 1995) are closer to each other than to Cebidae. This tree was only slightly preferred over the tree in which Pithecidae is closer to Cebidae than to Atelidae (Schneider et al. 1996). In either case, however, it seems clear that the 2 X-linked duplicate pigment genes were derived from a duplication in the common ancestor of howler monkeys, because only the genus *Alouatta* has the 2 duplicate genes. In other words, this duplication was independent of the one that produced the 2 X-linked pigment genes in humans, apes, and OWMs. Note that the single origin hypothesis, which assumes that the two-gene systems in howler monkeys and in humans, apes and OWMs were derived from the same duplication, implies the persistence of the 2 duplicate genes in the howler monkey lineage and a recent loss or degeneration of 1 of the 2 duplicate genes in the *Ateles-Lagothrix* lineage. In this case, the Southern blot analysis should be able to detect the degenerated duplicate, contrary to our observation of a single band. In summary, the independent origin hypothesis is more plausible than the single origin hypothesis.

Coding sequences

Since there are 2 critical amino acid residues in exon 5 (positions 277 and 285) that contribute to the spectral differences between the red and green pigments in primates (Nathans et al. 1986, Neitz et al. 1991), we first sequenced exon 5 to identify the sequences of the green- and red-sensitive pigment gene in the howler monkey. After this, exon 4, intron 4, and then exon 3 were sequenced using the strategy described in Materials and Methods. Since exon 4, intron 4, and exon 5 were amplified together, it was easy to determine the linkage of the amino acids at critical positions 229 (in exon 4), 277, and 285. The green pigment gene has isoleucine (I) at position 229, phenylalanine (F) at position 277, and alanine (A) at position 285. The red pigment gene has phenyalanine (F) at position 229, tyrosine (Y) at po-

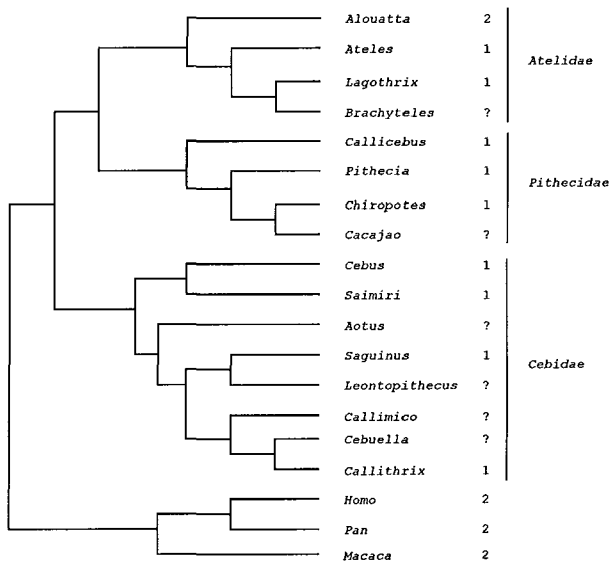

Fig. 2. Phylogenetic relationships between genera of New World monkeys and the number of copies of the X-linked color vision genes.

sition 277, and threonine (T) at position 285. The linkage of positions 229, 277, and 285 with the critical site at position 180 is less certain because this position is located in exon 3 and we did not succeed in amplifying exon 3, intron 3, and exon 4 all together. However, since the green and red pigments in the howler monkey have very similar spectral sensitivities to the human green and red pigments, respectively, and also to the squirrel monkey green and red pigment alleles (with spectral peaks at 535 and 562 nm), we assume that the alanine (A) found at position 180 is characteristic of the green pigment gene while the red pigment gene has serine (S).

Exons 3, 4, and 5 of the howler monkey pigment genes were compared with those of the human red and green pigment genes and with the 3 allelic sequences of each of 4 NWM species (*Saimiri sciureus, Callithrix jacchus, Cebus nigrivittatus*, and *Saguinus mystax*) (Figure 3). In these exons, 79 positions are variable, 25 of which result in amino-acid changes (including the 4 critical sites). For all comparisons there is no nucleotide position at which the sequence of the howler monkey is the same as a human sequence but different from any other NWM sequence. Conversely, at 8 positions, all NWM sequences (including the 2 howler monkey sequences) are identical but different from the 2 human sequences. It seems clear that the howler monkey coding sequences are closer to the NWM sequences than to the human sequences. Moreover, the 2 howler monkey sequences differ by only 6 positions in the 3 exons, in addition to the 4 critical sites. These results further support the independent origin of trichromacy in the howler monkey.

Intronic sequences

Intron 4 sequences of the 2 howler monkey genes were aligned with the human red (and identical green) pigment intron 4 sequence and with the 12 allelic sequences obtained from the 4 other NWM species studied (Shyue et al. 1995, Boissinot et al. unpubl. data). The alignment is 2005 base pair (bp) long and includes 498 variable sites and 93 insertions and deletions (indels). Kimura's 2-parameter distances were computed (Table 1). The divergences between the human sequence and the howler monkey sequences (12%) are on average twice the number of divergences between the howler monkey sequences and other NWM sequences (~6%). If we examine indels as phylogenetic characters, the NWM sequences have 13 indels in common, but the howler monkey and human sequences share no indels that are not found in 1 of the 12 NWM allelic sequences. Furthermore, all NWM sequences have an *Alu* insertion (from position 91 to 410 of the alignment) in common, which is not found in humans. Distances, indels, and the *Alu* insertion clearly indicate that the howler monkey sequences are closer to the other NWM sequences than to the human sequences. This result again supports the independent origin of trichromacy in the howler monkey.

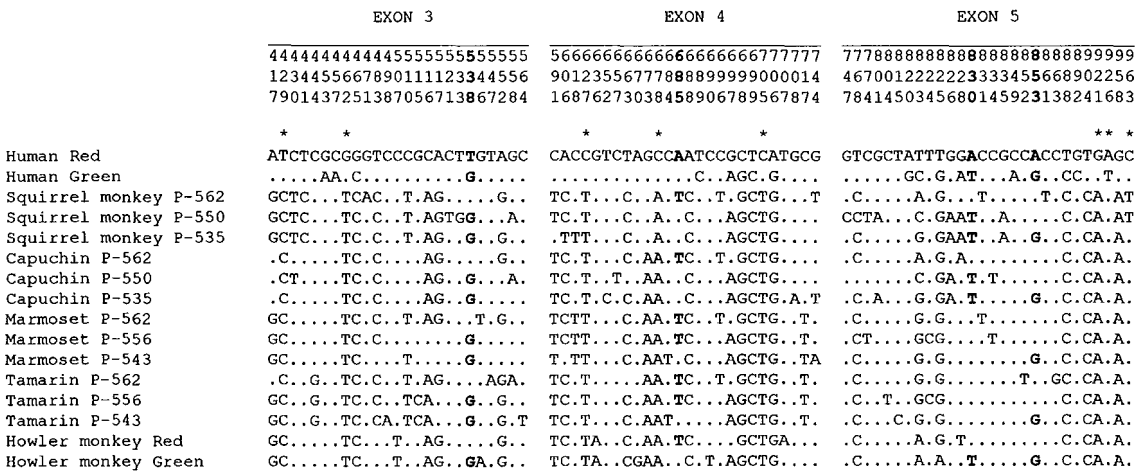

Fig. 3. Sites at which variation is observed among the 2 human genes, 12 allelic sequences in 4 NWM species, and the 2 howler monkey genes. The numbers at the top of the table refer to the positions of the site on the complete coding sequence. The 4 positions in bold face are the 4 critical amino acid residues: positions 180, 229, 277, and 285. The 8 positions with * at the top are the positions at which all NWM sequences are identical but different from the 2 human sequences.

Table 1. Number of nucleotide substitutions per 100 sites between intron 4 sequences

		Human	Squirrel monkey			Capuchin			Marmoset			Tamarin			Howler monkey	
		Red	P-562	P-550	P-535	P-562	P-550	P-535	P-562	P-556	P-543	P-562	P-556	P-543	Red	Green
Human	Red															
Squirrel	P-562	11.78 ± 1.1														
monkey	P-550	11.77 ± 1.1	2.17 ± 0.4													
	P-535	12.07 ± 1.1	2.16 ± 0.4	1.90 ± 0.4												
Capuchin	P-562	10.76 ± 1.0	4.13 ± 0.6	3.95 ± 0.6	4.13 ± 0.6											
	P-550	11.06 ± 1.5	4.13 ± 0.6	3.95 ± 0.6	4.31 ± 0.6	1.72 ± 0.4										
	P-535	11.07 ± 1.5	4.50 ± 0.6	4.32 ± 0.6	4.50 ± 0.6	1.46 ± 0.4	2.16 ± 0.4									
Marmoset	P-562	12.48 ± 1.1	6.72 ± 0.8	6.15 ± 0.7	6.62 ± 0.8	5.59 ± 0.7	5.87 ± 0.7	5.69 ± 0.7								
	P-556	12.26 ± 1.1	6.24 ± 0.8	6.05 ± 0.7	6.32 ± 0.8	5.68 ± 0.7	5.95 ± 0.7	5.77 ± 0.7	4.41 ± 0.6							
	P-543	12.18 ± 1.1	5.97 ± 0.7	5.87 ± 0.7	5.96 ± 0.7	5.50 ± 0.7	5.87 ± 0.7	5.97 ± 0.7	4.22 ± 0.6	3.50 ± 0.6						
Tamarin	P-562	11.85 ± 1.1	5.49 ± 0.7	5.58 ± 0.7	5.95 ± 0.7	5.58 ± 0.7	5.77 ± 0.7	5.96 ± 0.7	5.86 ± 0.7	5.49 ± 0.7	4.76 ± 0.6					
	P-556	11.67 ± 1.1	5.22 ± 0.7	5.13 ± 0.7	5.49 ± 0.7	5.31 ± 0.7	5.50 ± 0.7	5.69 ± 0.7	5.60 ± 0.7	5.03 ± 0.7	4.58 ± 0.6	1.29 ± 0.3				
	P-543	12.07 ± 1.1	5.49 ± 0.7	5.59 ± 0.7	5.77 ± 0.7	5.31 ± 0.7	5.31 ± 0.7	5.50 ± 0.7	4.86 ± 0.7	4.39 ± 0.6	4.58 ± 0.6	2.51 ± 0.5	2.25 ± 0.4			
Howler	Red	12.07 ± 1.1	6.34 ± 0.8	5.85 ± 0.7	6.33 ± 0.8	5.77 ± 0.7	5.96 ± 0.7	5.96 ± 0.7	6.80 ± 0.8	6.60 ± 0.8	6.61 ± 0.8	6.50 ± 0.8	6.33 ± 0.8	6.32 ± 0.8		
monkey	Green	12.29 ± 1.1	6.25 ± 0.8	5.77 ± 0.7	6.24 ± 0.8	5.68 ± 0.7	5.87 ± 0.7	6.06 ± 0.7	6.90 ± 0.8	6.98 ± 0.8	6.52 ± 0.8	6.41 ± 0.8	6.24 ± 0.8	6.61 ± 0.8	1.29 ± 0.3	

Means and standard errors were estimated using Kimura 2-parameters method. Gaps are not included in the comparison.

Phylogenetic analysis and evidence of gene conversion

The distances between intron 4 sequences were used to build a neighbor-joining tree (Fig. 4). A long branch leads from the human sequence to the NWM sequences (including the howler monkey sequences). The 3 polymorphic alleles in each species form a monophyletic group supported by a high bootstrap value (100%, 100%, 96%, or 99%). Similarly, the 2 howler monkey sequences strongly group together. The branching of the species-specific groups of sequences follows the known phylogenetic relationships (Fig. 2): the Cebidae species (squirrel monkey, capuchin, marmoset, and tamarin) form a monophyletic group, and within this group, the squirrel monkey is closer to the capuchin, whereas the marmoset is closer to the tamarin.

However, the clustering of the (allelic) intron 4 sequences in each species might be due to gene conversion (Boissinot et al. unpubl. data). Gene conversion has been found to have occurred between the 2 X-linked pigment genes in humans, chimpanzees, and baboons (Ibbotson et al. 1992, Winderickx et al. 1993, Shyue et al. 1994, Zhou and Li 1996), and between the X-linked pigment alleles in New World monkeys (Boissinot et al. unpubl. data). For this reason, it is quite possible that the tri-allelic systems in the squirrel monkey, the capuchin, the marmoset, and the tamarin have been derived from only 1 or 2 origins rather than the 4 origins shown in Figure 4 (Boissinot et al., unpubl. data).

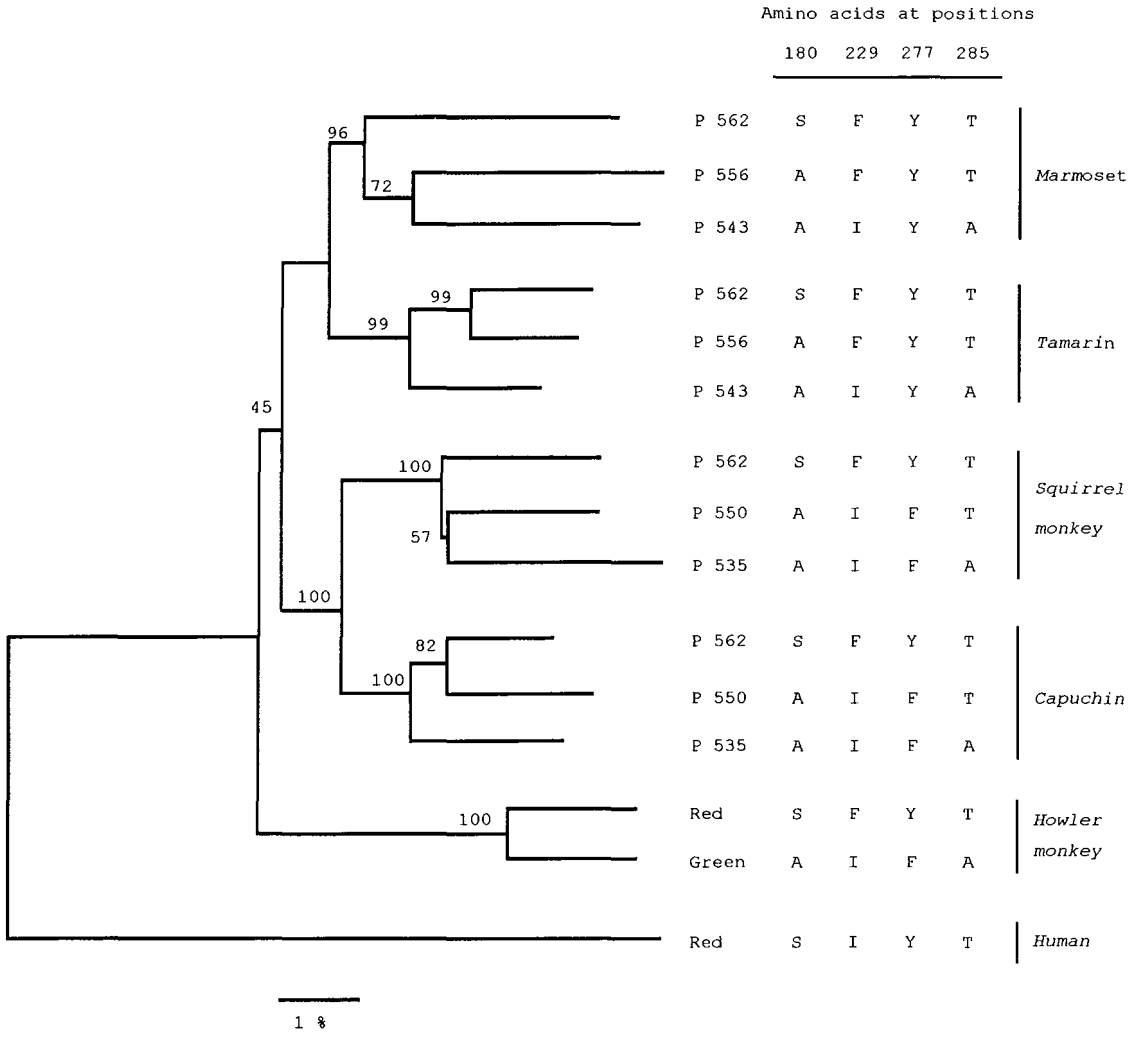

Fig. 4. Neighbor-joining tree derived from an analysis of intron 4 sequences. The number at each node denotes the proportion of 500 bootstrap replicates that supported the subset of sequences.

Similarly, the clustering of the howler monkey red and green pigment genes in Figure 4 might also be due to gene conversion. To examine this possibility, we plotted the sequence divergence between introns of the 2 genes along the sequence (Figure 5). The divergence (~1.6%) in the first 700 bp of intron 4 is 2.5 times lower than that in the region from position 700 to position 1100 (~4%). Moreover, the divergence in the region from position 1100 to the 3' end is close to 0%. This uneven distribution of divergence suggests that the 5' and 3' ends of intron 4 between the red and green genes were homogenized by recent conversion events, while the central portion was either not homogenized or homogenized by much earlier conversion events. As gene conversion seems to have occurred frequently, the phylogenetic relationships among these sequences are uncertain and need to be studied further.

Origin of trichromacy in the howler monkey

This study and that of Jacobs et al. (1996) strongly suggest that the trichromacy in the howler monkey arose from a relatively recent duplication of the X-linked pigment gene and arose independently of the trichromacy in humans, apes, and OWMs. Unfortunately, the origin of the 2 alleles that were incorporated into 1 chromosome to produce the duplication is difficult to infer because noncritical regions such as intron 4 could have been homogenized by gene conversion. However, we note that at the 4 critical sites for spectral tuning, the green and red pigments in the howler monkey are, respectively, identical to the P535 and P562 pigments in the squirrel monkey and capuchin (Fig. 4). Thus, it is possible that the green and red pigment genes in the howler monkey were derived by the incorporation of 2 alleles similar to the P535 and P562 alleles currently existing in the squirrel monkey and capuchin. This hypothesis requires that the substitutions at the 4 critical sites occurred only once and it does not require any parallel change. The alternative hypothesis (suggested by the tree in Figure 4) requires many parallel changes at the 4 critical sites. The former hypothesis is more parsimonious and seems more likely. However, more data are needed to draw a more definitive conclusion. If this hypothesis is correct, it implies that the P535 and P562 alleles existed before the divergence between the squirrel monkey and howler monkey lineages, i.e., before the divergence of the Cebidae and Atelidae from the ancestral NWM, which has been estimated to have occurred about 20 million years ago (Schneider et al. 1993). Such a long persistence would imply the maintenance of these alleles by some type of balancing selection (e.g., heterozygote advantage).

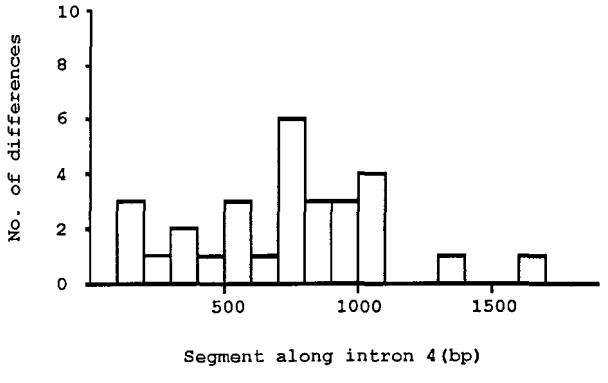

Fig. 5. Histograms showing variation in divergence between the 2 X-linked color vision genes of the howler monkey as a function of position in intron 4. Horizontally each bar represents a 100-bp segment. Vertically each bar shows the number of differences per segment.

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吼猴性染色體上複製色覺基因之來源及分子演化

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吼猴 (howler monkey) 為新世界猴子之一屬。跟人類及舊世界猴子一樣，吼猴可以辨別三種顏色，因為牠們擁有一個體染色體上 (藍色素蛋白質) 和兩個性染色體上 (紅和綠色素蛋白質) 的色覺基因。但是，其它已經詳細研究過的新世界猴子都只有一個體染色體上和一個性染色體上的色覺基因，只是牠們性染色體上色覺基因座上有三個對偶子 (alleles)。為瞭解吼猴三色覺基因的來源，我們研究了數種新世界猴子性染色體上色覺基因的數目，並將一雄性吼猴的 intron 4 和 exons 3，4 和 5 的核酸序列定序出來。利用 SSCP 及 Southern blotting 的技巧，我們證明了 spider monkey，woolly monkey，saki monkey 和 beared saki monkey 的性染色體上只有一個色覺基因。因此我們可以說，在新世界猴子中，只有吼猴擁有二個性染色體上的色覺基因。Exons 3, 4 和 5 及 intron 4 的核酸序列顯示吼猴性染色體上的兩個複製基因的來源與人類及舊世界猴子性染色體上的兩個基因的來源不同。其次，由胺基酸的序列可看出吼猴的兩個複製基因是分別由一個紅色素及一個綠色素同位子合併而來。這兩個同位子很類似現今存於 squirrel monkey 和 capuchin 中的紅色素及綠色素同位子。此外，吼猴的兩個基因的 intron 4 的核酸序列又顯示出晚近的 gene conversion 現象，以致這兩個 intron 4 變為很相似，這又進一步證明 gene conversion 的現象常發生於性染色體上的色覺基因之間。

關鍵詞： 吼猴，色覺基因，基因複製，核酸序列，古老的多樣性。

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