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Recent evolution of uniform trichromacy in a New World monkey

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Abstract

Until recently, New World primates were found to have a single M/L photopigment gene on the X-chromosome. This arrangement limits males to dichromatic, or monochromatic color vision. Only females who were heterozygous for the M/L gene were trichromatic. Recently, an exception has been discovered. Male howler monkeys appear to have more than one M/L pigment gene, and both genders are uniformly trichromatic. We characterized promoter regions corresponding to two M/L pigment genes in howlers. Comparison of DNA sequences with those of humans and three species of New World primate suggest a recent and independent acquisition of a second M/L gene locus in the howler.

Keywords: *Alouatta*; Photopigment genes; Trichromacy; Evolution; Color vision

1. Introduction

Trichromatic color vision is not universal among primates. Humans and other Old World primates are routinely trichromatic, having short (S), middle (M) and long (L) wavelength-sensitive cone photoreceptor cells in their retina. In contrast, color vision among the New World primates is more variable. The nocturnal monkey, *Aotus trivirgatus*, is monochromatic. In other species of New World monkeys, the males have dichromatic color vision, while there is variation in the females within a species; some females are dichromatic but more than one-half have trichromacy [1–3]. The genes that encode the M and L cone photopigments can explain the difference in color vision between the Old and New World primates. Trichromacy in the Old World primates is based on the presence of both M and L pigment genes on the X-chromosome. New World primates can have genes to encode multiple pigments that absorb in the middle to long wavelength region of the spectrum; however, they only have one cone pigment gene per X-chromosome [1–4]. Males, therefore, rely on one M/L type pigment gene, whereas only heterozygous females have two genes—one from each X-chromosome—to encode two different photopigments that absorb in the middle-to-long wavelengths.

A recent discovery by Jacobs et al. revealed that the New World monkey story was not complete [5]. Measurements of spectral sensitivity using the electroretinogram (ERG) indicated that male and female howler monkeys (*Alouatta*) had the photopigment basis for trichromacy. Molecular genetic results indicated the presence of both M and L exon 5 sequences in individual male and female howlers.

In order to enjoy trichromacy, the howlers must have complete genes to encode M and L photopigments and have the transcriptional machinery to direct the expression of the genes into separate cone populations. The promoter region of the M and L pigment genes may play a role in segregating the expression of M versus L. This DNA sequence has not been examined in New World primates. The presence of multiple promoters in the howler would provide further evidence for the genetic basis of trichromacy. Investigation of the howler DNA sequence compared to human may lend further insight into whether the howlers maintained Old World trichromacy or invented New World trichromacy.

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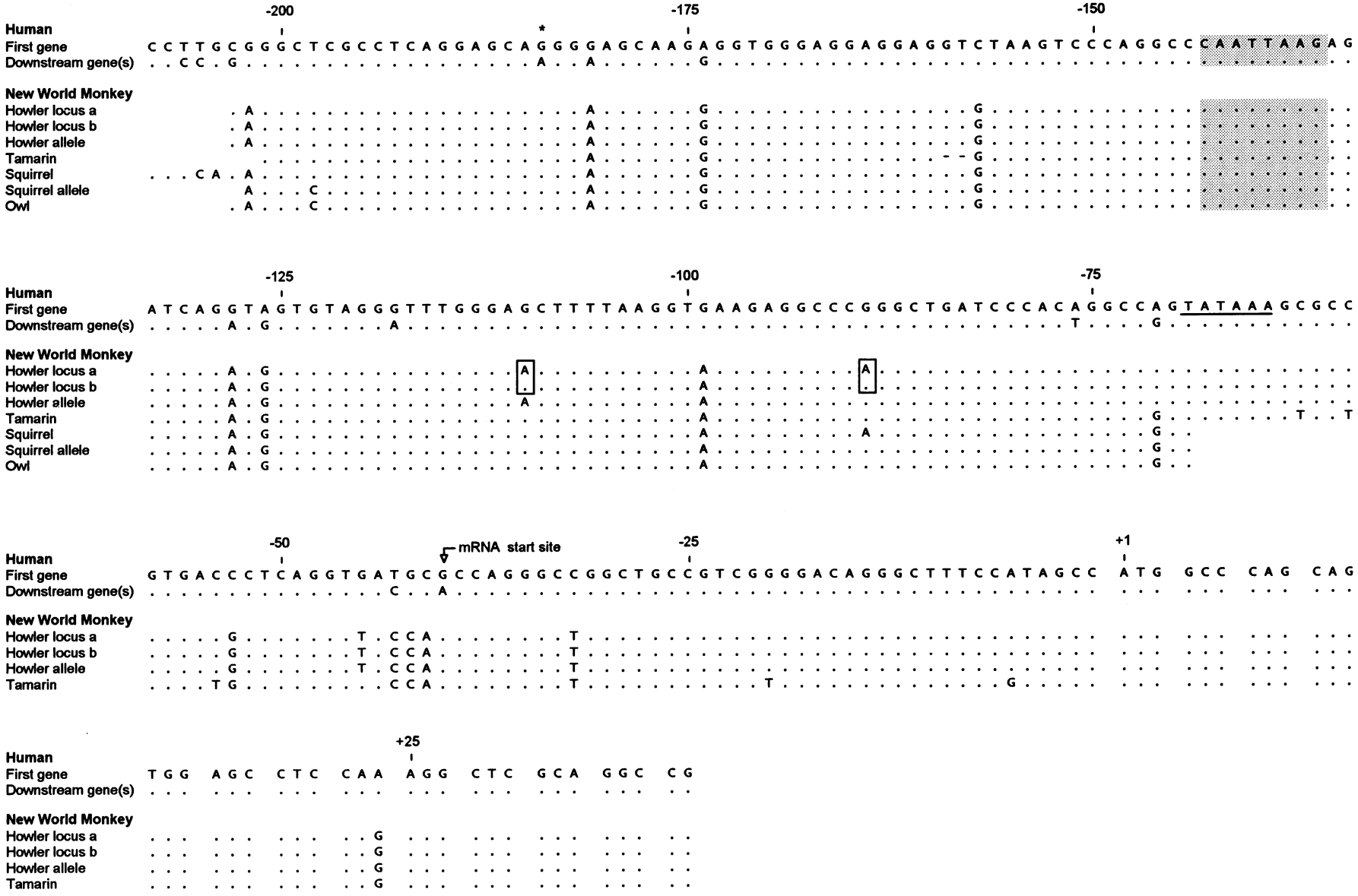

Fig. 1. (*caption opposite*)

2. Methods and materials

2.1. Subjects and DNA isolation

Genomic DNA was obtained from either whole blood or tissue using phenol/chloroform extraction method or Gene Releaser Bioventures) from the following New World monkeys: four male howler monkeys (one *Alouatta seniculus*, three *Alouatta caraya*), an owl monkey (*Aotus trivirgatus*), three squirrel monkeys (*Saimiri sciureus*) and a tamarin (*Saguinus fuscicollis*).

2.2. Amplification and sequencing of the promoter region of the X-chromosome visual pigment genes

The polymerase chain reaction (PCR) was used to amplify the promoter region of the X-chromosome visual pigment genes as described in Neitz and Neitz [6]. The DNA fragments amplified extended from base −230 to base −46 or from base −230 to base +68 (first nucleotide of exon 1 regarded as +1). The purpose of amplifying the longer DNA fragment from the howlers and the tamarin was to ensure that the region amplified was adjacent to pigment gene coding sequence, specifically the 5′ end of exon 1. The sequence of the 5′ primer used for both amplifications was 5′-CTGAGGGTCACGGCGCTTTAT-3′. The sequence of the 3′ primer used to amplify the shorter fragment was 5′-CCCAGCAAATCCCTCTGAGC-3′, and the sequence of the 3′ primer used to amplify the longer fragment was 5′-GGTGCTGTCCTCATAGCTGTCCTGCGGATG-3′. Amplified DNA fragments were gel purified on 5% polyacrylamide. Prior to gel purification, the electrophoresis apparatuses were treated with a solution of 10% bleach for 30 min to render any residual DNA unamplifiable [7]. The DNA bands were crushed and eluted in 100 μl TE 10 mM Tris–HCl, 1 mM EDTA, pH 7.8) at 68°C overnight. Twelve to 24 μl of the supernatant was used for sequencing, depending on the intensity of the DNA band when the ethidium bromide stained gel was exposed to UV light. The DNA was desalted and concentrated using an Amicon microconcentrator—30 and 100 μl of water. The Cycle Sequencing Kit (Perkin–Elmer) was used as recommended by the manufacturer to obtain DNA sequence.

2.3. Determination of haplotype

Restriction enzyme digest was used to determine how the sequence differences segregate in the howler loci. PCR products were digested with *Alu*I restriction enzyme to distinguish nucleotide A from G at position −110, and the *Sma*I restriction enzyme to distinguish nucleotide G from A at position −89. The fragments were electrophoresed on a 12% polyacrylamide gel and visualized using the FluorImager (Molecular Dynamics).

2.4. Sequence analysis

Cladograms were constructed using all the New World monkey and human promoter sequences to test different hypotheses about the evolution of trichromacy in the howler. Our analysis included nucleotide −200 through nucleotide −50. The MacClade cladistic analysis [8] was used to measure the degree of parsimony for each tree that was constructed to test each hypothesis. The bovine promoter sequence [9] was used as an outgroup to add information to the trees with regard to the DNA sequence for the ancestral pigment gene promoter. Tree 'length' indicated the total number of nucleotide replacements required for a particular phylogenetic tree.

3. Results

3.1. DNA sequence determination of the promoter region of M/L pigment genes in New World monkeys

Fragments containing the X-linked visual pigment gene promoter regions were amplified from genomic DNA of each New World monkey and directly sequenced. In Fig. 1, the sequencing results for the promoter regions are shown aligned to the corresponding promoter region from the first gene in the human X-chromosome array [10]. As a group, the promoter regions from the New World primates were more similar to one another than any of them was to the corresponding region of the first gene or the downstream genes in humans.

3.2. Confirmation of multiple promoter regions in the X-chromosome pigment genes of the howler

Sequences for two promoter regions were found in every howler, while all other New World monkeys had just one sequence. Two *A.caraya* and the *A.seniculus* had two promoter regions that differed at positions −110 and −89. A mixture of nucleotide G and A was

Fig. 1. Nucleotide sequences of promoter regions of the X-chromosome visual pigment genes from human, howler monkeys, a tamarin, squirrel monkeys and an owl monkey. Dots indicate identity to the corresponding nucleotide in the promoter region of the human first gene, which was determined by Nathans et al. [10]. Blanks indicate not sequenced and dashes indicate deletions. The first nucleotide of the translation start codon is labeled +1. TATA box is underlined. The PCE-1 core is highlighted. Boxes show the two locations at which the howler loci differed. Asterisk indicates the nucleotide position involved in tree length difference between Tree B and Tree C in Fig. 2.

found at each location. Restriction enzyme digest was used to determine how the sequence differences segregate in the howler loci. Fig. 1 shows each as 'Howler locus a' and 'Howler locus b' respectively. The restriction digest assay and DNA sequencing showed that the third *A.caraya* had evidence for locus 'b' and a 'Howler allele' A at position −110 and a G at position −89).

A second PCR amplification was performed to extend the sequence examined in the howlers into the coding sequence of the visual pigment gene. The same 5′ primer was paired with a 3′ primer homologous to a region in exon 1. The PCR product was the predicted size, and included DNA sequence corresponding to exon 1 from human. The first 38 base pairs of exon 1 in the howlers and the tamarin were determined and found to be identical to the human sequence, with the exception of one silent substitution at position +24.

The regions of DNA that were generally conserved among all subjects were also conserved in each howler locus. The largest stretch of conserved sequence contains a transcription factor-binding site believed to play a role in retina-specific expression, shown highlighted in Fig. 1. This binding site, called the photoreceptor conserved element (PCE-1), is conserved in the fruit fly and the mouse [11]. It appears that this motif is conserved in the primate lineage. Our data also suggest that the extended primate PCE-1 is 28 base pairs in length.

3.3. Cladistic analysis

The promoter DNA sequences in Fig. 1 were analyzed using the MacClade cladistic program to determine the relative parsimony of alternative hypotheses regarding the evolution of trichromacy in the howler. The region of promoter used in the analysis was from nucleotide −200 to nucleotide −50. All cladograms were rooted with the bovine M-L promoter sequence. Full trees including all the New World monkeys in our study were used to determine the differences in tree length (measurement of parsimony) for each hypothesis. For tree construction, we used the proposed phylogeny for the New World monkeys from [12]. Though the phylogeny of the New World primates is controversial, many of the groupings of species are straightforward and agreed upon. The monkeys in our analysis were among the latter with the exception of the owl monkey. In our study however, changing the placement of *Aotus* in the NW branch did not influence the difference in parsimony between hypotheses.

For simplification of illustration, the full trees were trimmed to include only the howler and the human branches (Fig. 2). The sites that were informative in the larger analysis with regard to differentiating the specific hypotheses are shown. The tree lengths in Fig. 2 reflect changes at these sites. Tree A illustrates the evolutionary scenario in which the two howler loci share common ancestors with the two human genes. In this case, the idea would be that the second howler locus was not acquired independently, rather, the two genes would have been maintained from an earlier event common to both New and Old World primates. There are two possible arrangements for this hypothesis (e.g. each howler locus paired with either the human first gene or the human downstream gene). Neither possibility was as parsimonious as tree B or C (Fig. 2) which correspond to the hypothesis that the howler acquired the additional locus independently. The most parsimonious tree that could be constructed under the hypothesis of a common origin had a length of 5 compared to a length of 1 for tree C, the most parsimonious one that could be constructed under the hypothesis that howler trichromacy evolved independently.

4. Discussion

The finding of two different promoter regions taken together with the evidence for both M and L exon 5 sequences is consistent with the presence of two complete genes underlying trichromacy in the howler. A high degree of similarity exists among all the New World monkey sequences. However, only *Alouatta* showed evidence for multiple promoters.

The howler promoter loci are very similar in sequence suggesting that this New World monkey acquired the additional pigment gene locus recently; within the same promoter region, there are 10 base changes between the human M and L genes and only one or two base changes between the howler genes. The recent gene acquisition in the howler would imply that the event was independent from the gene duplication that gave rise to trichromacy in the Old World primates. Cladistic analysis, using all the New World monkey and the human promoter sequences, provided further support for this conclusion. The phylogenetic trees representing the hypothesis that howlers acquired a second locus in an independent event require three or four fewer nucleotide replacements than the alternatives in which the two howler loci share a common origin with the two loci of the Old World primates. This is strong evidence in favor of an independent origin for the two loci of the howler.

There is one final point. It is often assumed that the Old World primates acquired their second locus after the New and Old World primates diverged. We did not make this assumption in our analysis. Instead, the trees B and C both represent an independent origin for the howler second locus but C places the acquisition of the Old World second locus before, and B places it after division of the New and Old World lineages. Interestingly, tree C requires one fewer nucleotide change than tree B. Of course, no strong conclusion can be drawn

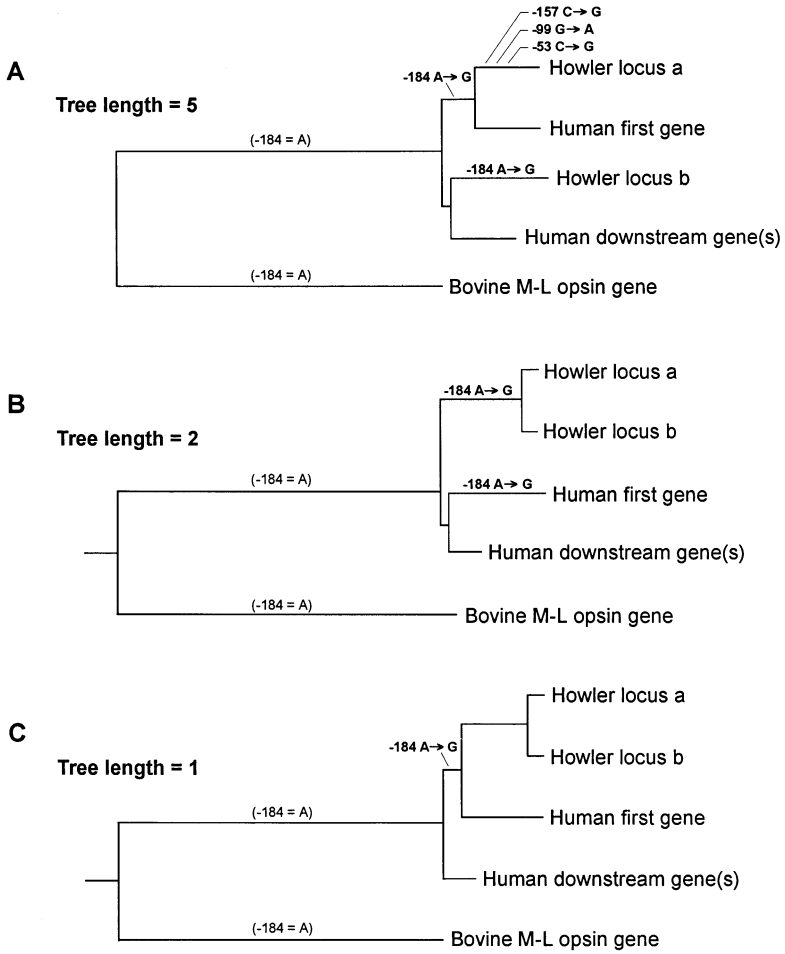

Fig. 2. Three phylogenetic trees used to test hypotheses regarding the evolution of trichromacy in the howler. Cladistic analysis was performed using nucleotides −200 through −50 for the sequences shown in Fig. 1. To simplify illustration, the trees were trimmed to include only the human, howler and bovine branches. The tree lengths for the full cladograms were much larger than those indicated. However, the relative differences between the large trees are preserved in the tree lengths shown for the trimmed trees in the figure. With regard to the hypotheses tested using the full cladograms, only the informative nucleotide positions are indicated. Tree lengths reflect changes at these positions.

from a single site. However, it can serve as reminder that the hypothesis has not been ruled out in which ancestors to modern New and Old World primates had second locus but the New World primates lost it early in their evolution after the division of the lineages.

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