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BRIEF REPORT

Opsin Gene Polymorphism Predicts Trichromacy in a Cathemeral Lemur

CARRIE C. VEILLEUX* AND DEBORAH A. BOLNICK
Department of Anthropology, University of Texas at Austin, Austin, Texas

Recent research has identified polymorphic trichromacy in three diurnal strepsirrhines: Coquerel's sifaka (*Propithecus coquereli*), black and white ruffed lemurs (*Varecia variegata*), and red ruffed lemurs (*V. rubra*). Current hypotheses suggest that the transitions to diurnality experienced by *Propithecus* and *Varecia* were necessary precursors to their independent acquisitions of trichromacy. Accordingly, cathemeral lemurs are thought to lack the M/L opsin gene polymorphism necessary for trichromacy. In this study, the M/L opsin gene was sequenced in ten cathemeral blue-eyed black lemurs (*Eulemur macaco flavifrons*). This analysis identified a polymorphism identical to that of other trichromatic strepsirrhines at the critical amino acid position 285 in exon 5 of the M/L opsin gene. Thus, polymorphic trichromacy is likely present in at least one cathemeral *Eulemur* species, suggesting that strict diurnality is not necessary for trichromacy. The presence of trichromacy in *E. m. flavifrons* suggests that a re-evaluation of current hypotheses regarding the evolution of strepsirrhine trichromacy may be necessary. Although the M/L opsin polymorphism may have been independently acquired three times in the lemurid–indriid clade, the distribution of opsin alleles in lemurids and indriids may also be consistent with a common origin of trichromacy in the last common ancestor of either the lemurids or the lemurid–indriid clade. Am. J. Primatol. 71:86–90, 2009.

Key words: strepsirrhines; color vision; cathemerality; *Eulemur macaco flavifrons*; opsin genes; primate evolution

INTRODUCTION

Trichromacy is unique to primates within placental Mammalia and was believed to be limited to anthropoids [Jacobs, 1993] until recently. Catarrhine trichromacy is achieved through interactions between three cone types (short-wavelength-sensitive S cones, medium-wavelength-sensitive M cones, and long-wavelength-sensitive L cones), which are controlled by the autosomal S opsin gene and the X chromosome M and L opsin genes, respectively [Hunt et al., 2005; Nathans et al., 1986]. Rather than having separate M and L opsin genes, most platyrrhines maintain a single M/L opsin gene on the X chromosome that manifests multiple alleles differing in peak spectral sensitivity from 530 to 562 nm [polymorphic trichromacy: Surridge et al., 2003]. This polymorphism at the M/L opsin locus permits trichromacy in heterozygous females, whereas homozygous females and hemizygous males are dichromats.

In contrast to its wide distribution in platyrrhines, polymorphic trichromacy is rare in strepsirrhines [Jacobs & Deegan, 2003a]. Lorisiforms have only S cones and are monochromats, whereas most lemurs are dichromats [Dkhissi-Benyahya et al., 2001; Jacobs et al., 1996; Kawamura & Kubotera, 2004; Perry et al., 2007; Tan & Li, 1999; Tan et al., 2005]. In a survey of prosimian opsin genes, Tan and Li [1999] found a polymorphism in the M/L opsin gene at amino acid site 285 in two diurnal lemur species (*Propithecus coquereli* and *Varecia rubra*). This polymorphism was expected to result in a spectral shift of 15 nm (~543 to 558 nm) for the resulting opsin [Tan & Li, 1999]. Jacobs and colleagues verified the presence of distinct 545 nm-sensitive and 558 nm-sensitive cones in *P. coquereli* and *V. variegata* using electroretinogram flicker photometry [Jacobs et al., 2002; Jacobs & Deegan, 2003b]. *Varecia* and *Propithecus* are believed to have acquired polymorphic trichromacy independently [Jacobs & Rowe, 2004; Surridge et al., 2003].

The diurnal activity pattern exhibited by these trichromatic lemurs has led some authors to suggest diurnality is necessary for the evolution of lemur polymorphic trichromacy [Jacobs & Deegan, 2003b].

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*Correspondence to: Carrie C. Veilleux, Department of Anthropology, University of Texas at Austin, 1 University Station C3200, Austin, TX 78712-0303.
E-mail: cveilleux@mail.utexas.edu

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However, not all diurnal lemurs exhibit the potential for polymorphic trichromacy. Jacobs and colleagues have identified only one M/L cone type in a sample of 17 *Lemur catta*, suggesting that allelic variation at the M/L opsin locus is unlikely in this species [Blakeslee & Jacobs, 1985; Jacobs & Deegan, 1993, 2003a]. Jacobs and Deegan [2003a] offered three explanations for dichromacy in *Lemur*: (1) the necessary mutations may not have occurred by chance, (2) factors in addition to diurnality may be necessary for the evolution of the trait, and (3) *Lemur* is not strictly diurnal. A number of lemur species are cathemeral, or active throughout the light and dark periods of a 24-hr cycle [Curtis & Rasmussen, 2002; Tattersall, 1987], and some authors suggest *L. catta* may be cathemeral [Pereira, 1995; Traina, 2001]. Because cathemeral lemurs have visual systems balancing the mutually exclusive needs for sensitivity in low light and acuity in high light [Kirk, 2006; Peichl et al., 2001], cathemeral visual anatomy may not support the evolution of trichromacy [Jacobs & Deegan, 2003a].

However, the presence of trichromacy in cathemeral marsupials [Arrese et al., 2002, 2006] suggests that cathemerality and trichromacy can be compatible. Furthermore, research into cathemeral lemur color vision has been limited. A behavioral color discrimination test suggests that *Eulemur macaco* may be trichromatic [Gosset & Roeder, 2000], although similar results in *L. catta* were attributed to interaction between S cones, M cones, and rods [Blakeslee & Jacobs, 1985]. Measurements of cone spectral sensitivities for three *E. macaco* and four *E. fulvus* found only one cone type with λ_{max} at ~543 nm [Bowmaker, 1991; Jacobs & Deegan, 1993]. Analyses of the M/L opsin gene for one *E. fulvus* and two *E. mongoz* also found only a single 543 nm allele [Tan & Li, 1999]. Here, we expand lemur opsin gene research to include a new cathemeral species, the blue-eyed black lemur (*E. macaco flavifrons*).

METHODS

The M/L opsin gene was studied in ten blue-eyed black lemurs (seven females and three males). Banked blood samples were acquired from nine individuals at the Duke Lemur Center. A blood sample from a female housed in the Animal Resource Center at the University of Texas at Austin was also obtained. Because this sample was drawn for routine medical care under veterinary supervision, approval from the University of Texas Institutional Animal Care and Use Committee was unnecessary (G. Otto, personal communication). Genomic DNA was isolated from the samples using the DNeasy Tissue and Blood Extraction Kit (Qiagen). In other lemurs, the critical tuning position in the M/L opsin gene is at amino acid site 285 in exon 5, so we sequenced exon 5 from our samples using strepsirrhine-specific primers provided by Y. Tan. Each PCR contained 3 μL DNA template, 16 μL ddH_20, 2.5 μL PCR enhancer (Epicentre), 2.5 μL Hotmaster Taq buffer with $MgCl_2$ (5 Prime), 0.45 μL 10 μM dNTPs, 0.45 μL 20 μM primers, and 0.1 μL Hotmaster Taq, for a total

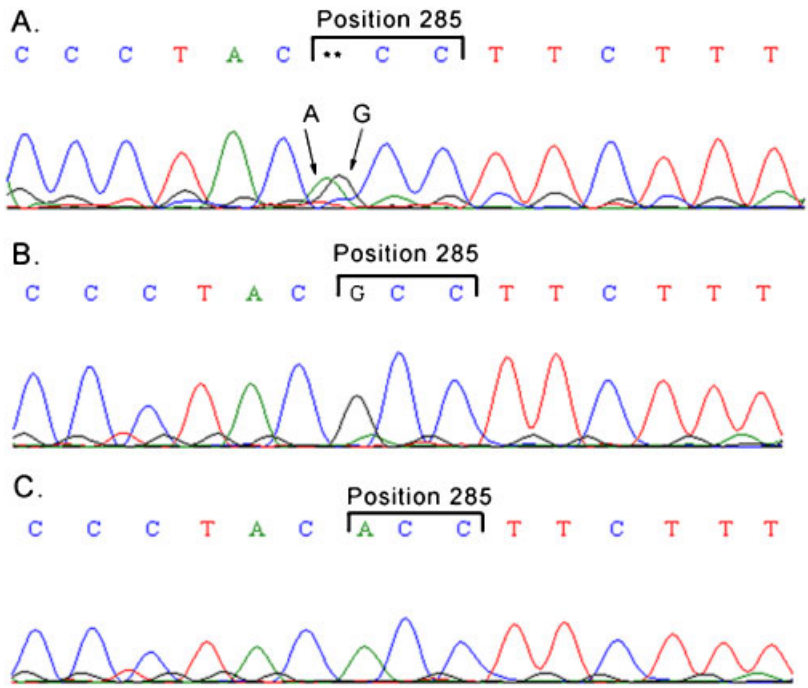

Fig. 1. M/L opsin gene sequences traces. Sequence traces of the critical amino acid position for one polymorphic female **(A)** and her two male offspring. One **(B)** inherited the Alanine allele (codon GCC), whereas the other **(C)** inherited the threonine allele (codon ACC).

volume of 25 μL. The PCR conditions were: (1) a hold for 2 min at 94°C; (2) 30 cycles of 30 sec at 94°C, 30 sec at 57°C, and 1 min at 65°C; (3) a hold for 6 min at 65°C. PCR products were purified with the QIAquick PCR Purification Kit (Qiagen) and sequenced using an ABI 3130 DNA Analyzer at the University of Texas DNA Sequencing Facility. Sequences were analyzed in BioEdit 7.0.9.0 [Hall, 1999] and compared with human [Deeb et al., 1994] and *Propithecus* sequences from GenBank. PCR products for two polymorphic females were cloned using a TOPO TA Cloning Kit (Invitrogen).

RESULTS

Of the ten lemurs analyzed, two females were heterozygous at the M/L locus and expressed a polymorphism at amino acid site 285 (Ala/Thr) identical to that seen in *Propithecus* and *Varecia*. Cloning confirmed the presence of distinct alanine and threonine alleles. Two of the males were offspring of one polymorphic female, and each inherited a different allele from their mother (Fig. 1). The remaining lemurs (five females and one male) exhibited the threonine allele. Sequences for these *Eulemur* samples have been deposited in GenBank (GenBank accession # FJ228726).

DISCUSSION

This study offers the first evidence of polymorphic trichromacy in a cathemeral primate. Jacobs et al. [2002] confirmed that the observed M/L opsin polymorphism was directly linked to the presence of distinct cone types in *Varecia* and *Propithecus*. They found that individuals with the alanine allele exhibited ~543 nm-sensitive cones (λ_{max} = 544–546 nm), whereas individuals with the threonine allele exhibited ~558 nm-sensitive cones (λ_{max} = 557–559 nm). Thus, in this sample of *E. macaco flavifrons*, two females possess 543 nm- and 558 nm-sensitive cones, one male possesses 543 nm-sensitive cones, and two males and five females possess 558 nm-sensitive cones. Definitive evidence

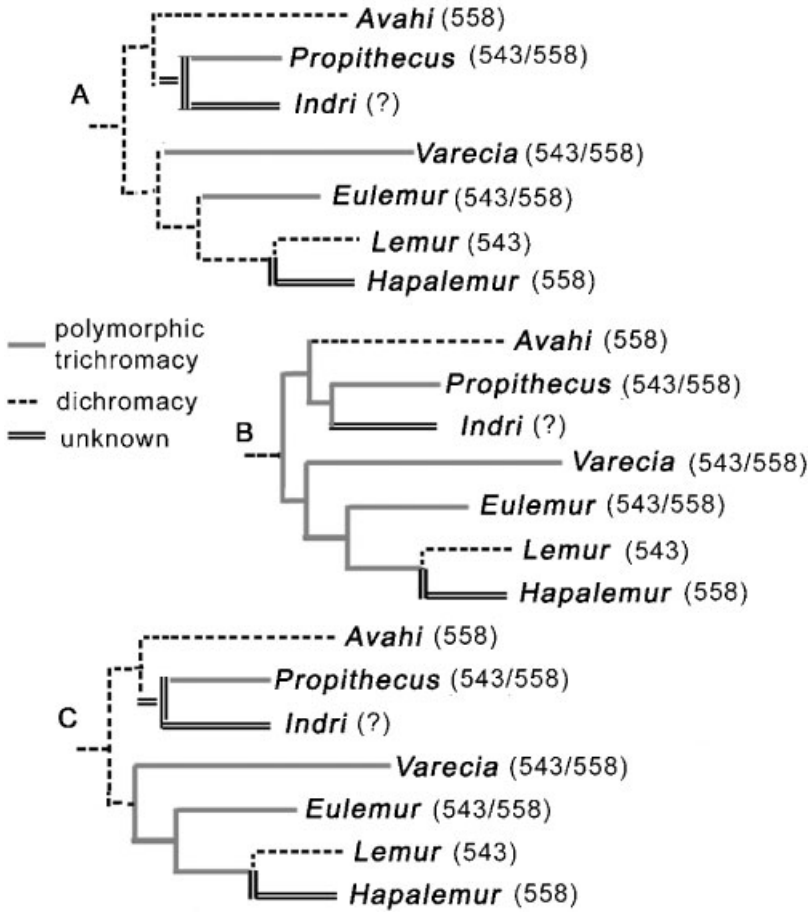

Fig. 2. Hypotheses for the evolution of polymorphic trichromacy in lemurs. Solid lines indicate polymorphic trichromacy, dashed lines indicate dichromacy, and double lines indicate unknown opsin allele distributions. Numbers next to genera indicate opsin alleles (543 nm-sensitive or 558 nm-sensitive) known for that genus. Phylogeny adapted from Roos et al. [2004]. **(A)**: Three independent origins of trichromacy, in *Propithecus, Varecia,* and *Eulemur*. **(B)**: Trichromacy evolved in the lemurid–indriid common ancestor and was later lost in *Avahi*, *Lemur*, and possibly *Hapalemur*. **(C)**: Trichromacy independently evolved at the base of the lemurid family and in *Propithecus*. As in hypothesis B, trichromacy was later lost in *Lemur* and possibly *Hapalemur*.

of trichromacy in this species awaits behavioral confirmation. However, because strepsirrhines likely possess neural mechanisms to process trichromatic color cues [Jacobs et al., 2002, 2007; Yamada et al., 1998], it is reasonable to suggest that blue-eyed black lemurs are polymorphic trichromats. The results of this study contradict previous suggestions that strict diurnality is necessary for trichromacy to be acquired or maintained in lemurs [Jacobs & Deegan, 2003a] and are consistent with evidence of routine trichromacy in cathemeral marsupials [Arrese et al., 2002, 2006].

Although previous discussions of lemur trichromacy implied that the M/L opsin polymorphism emerged independently in *Varecia* and *Propithecus* [Jacobs & Rowe, 2004; Surridge et al., 2003], its presence in *Eulemur* suggests that a re-evaluation of this scenario may be necessary. Molecular studies have had difficulty resolving lemur family relationships, but many studies, including a recent analysis of short interspersed elements (SINEs), have supported a Lemuridae–Indriidae sister relationship [DelPero et al., 2001, 2006; Roos et al., 2004]. SINEs have extremely low probabilities of homoplasy and thus represent a more stable evolutionary signal than some other markers [DelPero et al., 2006; Roos et al., 2004; Xing et al., 2007]. Given Roos et al.'s [2004] phylogeny and the observed distribution of opsin alleles in lemurids and indriids, three hypotheses exist for the origin of lemur polymorphic trichromacy (Fig. 2). Hypothesis **A** represents the traditional view, whereas **B** places the emergence of trichromacy at the base of the lemurid–indriid clade and **C** depicts the independent acquisition of trichromacy by the lemurid last common ancestor (LCA) and *Propithecus*. Evaluation of these hypotheses is currently limited by lack of data on lemur opsin allele distributions. *Hapalemur* is represented by a single male [Tan & Li, 1999] and there are no published data on *Indri* opsin alleles. Intriguingly, Roos et al.'s, [2004] reconstruction of the lemurid–indriid LCA as diurnal or cathemeral is consistent with a lemurid–indriid origin of trichromacy. As both diurnality and cathemerality are compatible with polymorphic trichromacy, a cathemeral or diurnal LCA would offer an opportunity for trichromacy to emerge at this node. If the ancestral lemurid–indriid condition was polymorphic trichromacy, the ecological conditions influencing the loss of trichromacy in some lineages, especially diurnal *L. catta*, should be further explored.

In conclusion, we have identified genetic evidence of polymorphic trichromacy in *E. macaco flavifrons*, a cathemeral genus previously considered dichromatic. This result has important implications for research on *Eulemur* behavior and ecology. Additionally, this study suggests that we may need to reassess our understanding of the factors involved in the evolution of polymorphic trichromacy in lemurs and its maintenance or loss in later lineages.

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