Signatures of Functional Constraint at Aye-aye Opsin Genes: The Potential of Adaptive Color Vision in a Nocturnal Primate

George H. Perry,*† Robert D. Martin,‡ and Brian C. Verrelli*†

*Center for Evolutionary Functional Genomics, The Biodesign Institute and School of Life Sciences, Arizona State University, Tempe; †School of Human Evolution and Social Change, Arizona State University, Tempe; and ‡Department of Anthropology, The Field Museum, Chicago, IL 60605

While color vision perception is thought to be adaptively correlated with foraging efficiency for diurnal mammals, those that forage exclusively at night may not need color vision nor have the capacity for it. Indeed, although the basic condition for mammals is dichromacy, diverse nocturnal mammals have only monochromatic vision, resulting from functional loss of the short-wavelength sensitive opsins gene. However, many nocturnal primates maintain intact two opsins genes and thus have dichromatic capacity. The evolutionary significance of this surprising observation has not yet been elucidated. We used a molecular population genetics approach to test evolutionary hypotheses for the two intact opsins genes of the fully nocturnal aye-aye (Daubentonia madagascariensis), a highly unusual and endangered Madagascar primate. No evidence of gene degradation in either opsins gene was observed for any of 8 aye-aye individuals examined. Furthermore, levels of nucleotide diversity for opsins gene functional sites were lower than those for 15 neutrally evolving intergenic regions (> 25 kb in total), which is consistent with a history of purifying selection on aye-aye opsins genes. The most likely explanation for these findings is that dichromacy is advantageous for aye-ayes despite their nocturnal activity pattern. We speculate that dichromatic nocturnal primates may be able to perceive color while foraging under moonlight conditions, and suggest that behavioral and ecological comparisons among dichromatic and monochromatic nocturnal primates will help to elucidate the specific activities for which color vision perception is advantageous.

Introduction

The evolution of color vision systems in primates has been extensively studied, including how adaptive differentiation in sensory perception is reflected in photopigment opsins genetic diversity (Shyue et al. 1995; Zhou and Li 1996; Nei et al. 1997; Verrelli and Tishkoff 2004). Many lemurs and New World monkeys have dichromacy, resulting from expression of one autosomal short-wavelength opsins (S-opsin) gene and one X-linked either medium-wavelength opsins (M-opsin) or long-wavelength opsins (L-opsin) gene (Jacobs 1996). Interestingly, there is an X-linked balanced polymorphism that maintains both M- and L-alleles in some species, such that only heterozygous females have trichromacy (Tan and Li 1999; Jacobs et al. 2002; Surridge and Mundy 2002). The X-linked opsins was duplicated independently in the common ancestor of caracalines (Old World monkeys, apes, and humans) and in the New World howler monkeys, facilitating full trichromacy in these primates for males and females (Nathans et al. 1986; Jacobs et al. 1996). Variation in color vision systems among primates may be tightly linked to evolutionary changes in foraging behavior among species (Martin and Ross 2005). For example, it is widely believed that color vision perception is adaptively correlated with foraging efficiency in detecting ripe fruits or immature, more readily digestible leaves (Allen 1879; Mollon 1989; Dominy and Lucas 2001). Foraging behaviors differ among primates not only in the types of food resources sought, but also in when foraging occurs. In fact, many primate species are nocturnal and forage exclusively at night (Sussman 1999), possibly without the need or capacity for color vision. Therefore, while trichromacy may be adaptive for diurnal primates, selective pressures on the visual systems of nocturnal foragers may be completely different.

If dichromacy becomes unnecessary coincident with nocturnal behavior, then functional constraint for at least one opsins gene may be relaxed, and the neutral fixation of gene disruptive mutations (i.e., stop codon, frameshift, or splice site mutations) can lead to monochromacy (Bowmaker and Hunt 2006). This process is consistent with the functional loss of the S-opsin in some nocturnal non-primate mammals, including raccoons, kinkajous, Syrian golden hamsters, African giant rats, and flying squirrels (Jacobs and Deegan 1992; Peichl and Moutairou 1998; Calderone and Jacobs 1999; Carvalho et al. 2006). Among primates, disruptive mutations in the S-opsin gene have occurred independently in at least three nocturnal lineages (Jacobs et al. 1993; Jacobs et al. 1996; Kawamura and Kubotera 2004; Tan et al. 2005). However, surprisingly, the S- and M/L-opsin genes reportedly remain intact and have molecular signatures consistent with long-term purifying natural selection in multiple nocturnal primates (Tan et al. 2005), which raises questions about the function of opsins and their role in the evolutionary history of nocturnality (see figure 1).

Two alternative interpretations of the presence of two intact opsins genes in some nocturnal primates are possible. First, that nocturnal habits necessarily lead to monochromacy over the long term, such that the presence of two functional opsins genes would indicate a relatively recent shift to nocturnality from a diurnal ancestor (Tan et al. 2005). Second, there may be long-term functional constraint on S- and M/L-opsin genes despite a persistent nocturnal activity pattern. If the latter is true, then dichromacy may be adaptive in some nocturnal primates, or opsins may have a function other than color vision (e.g., Nei et al. 1997). Here, we have conducted the first molecular population genetic study of the two intact opsins genes (Tan et al. 2005) in a fully nocturnal primate, the aye-aye.
*Daubentonia madagascariensis* of Madagascar. Aye-ayes are considered endangered by the World Conservation Union and are unquestionably one of the most phenotypically unusual primates, with continuously-growing incisors used to gnaw through bark and hard-shelled fruits and nuts, an elongated middle finger used to extract insects and fruit pulp, and the largest relative brain size of strepsirrhine primates (Cartmill 1979; Martin 1990; Sterling 1994; Fleagle 1999). Our population genetic analyses of this unique primate provide us with a rare opportunity to investigate how nocturnality and color vision may be intimately related in primate evolution.

(Materials and Methods)

Aye-aye Samples

Whole blood or liver tissue samples were obtained from 8 wild-born or unrelated aye-ayes: 7 wild-born individuals from Duke Lemur Center (2 individuals were captured from near Anjiamanigirana in northwest Madagascar, 3 from the Mananara Preserve in northeast Madagascar, and 2 from near Ankorabe in south Madagascar), and 1 captive-born animal from the Jersey Zoo (both parents were wild-born and captured from different locations in or near the Mananara Preserve in northeast Madagascar). Genomic DNA was extracted using standard protocols (Sambrook and Russell 2001). With respect to sample size, one cannot readily obtain DNA from wild aye-ayes, and there are few captive individuals. Therefore, our sample is a collection of available unrelated individuals from geographic locations across Madagascar. Our genetic analyses cannot address questions about population structure per se; nonetheless, these diversity estimates still reflect the aye-aye “population” as a whole and are the first of their kind.

Intergenic Regions Amplification and Sequencing

To test hypotheses about recent changes in functional constraint on aye-aye opsin genes, we need a sample of nucleotide diversity that reflects typical baseline levels of genetic variation in the aye-aye nuclear genome. For humans and most model organisms, population genetic data exist for many genes and therefore estimates of “neutral” variation are available as proxies to make inferences about functional constraint. However, owing largely to their endangered status and isolated geographic range, other than...
a study of the MHC locus (Go et al. 2005), we have no estimates of nuclear genome population genetic diversity for aye-ayes. Therefore, we first collected nucleotide sequence data from intergenic regions located throughout the aye-aye genome. Intergenic regions were initially selected from bacterial artificial chromosome (BAC) sequences from the small-eared galago (Otolemur garnettii) and ring-tailed lemur (Lemur catta) publicly available in GenBank through the ENCODE and Comparative Vertebrate Sequencing Projects (ENCODE Project Consortium 2004; Margulies et al. 2005). These represent sequences of taxa most closely related to the aye-aye, having diverged ~60 MYA (Yoder and Yang 2004). A BLAST analysis (Altschul et al. 1990) to the human genome sequence (Build 35) enabled omission of regions with putative gene identity (including exons and introns) as well as regions that contained repetitive elements. To avoid ascertainment bias related to highly conserved regions, we randomly sampled among these intergenic regions, ignoring the level of between-species nucleotide sequence conservation.

PCR primers were designed from lemur and galago sequences to amplify 2–3 kb fragments initially from one aye-aye individual. These initial fragments were cloned with the TOPO-XL PCR Cloning Kit (Invitrogen, Carlsbad, CA), and amplified and sequenced with vector primers. PCR products were purified using shipping shrimp alkaline phosphatase and exonuclease I (US Biochemicals) and nucleotide Sequencing kit and 3730 automated sequencer (Applied Biosystems, Foster City, CA), and amplified and sequenced with vector primers. PCR primers were designed from lemur and galago sequences to amplify 2–3 kb fragments initially from one aye-aye individual. These initial fragments were cloned with the TOPO-XL PCR Cloning Kit (Invitrogen, Carlsbad, CA), and amplified and sequenced with vector primers. PCR products were subsequently BLASTed to identify any putative gene regions or repetitive elements as above, in which case they were omitted, until 15 fragments passed our criteria. Aye-aye-specific primers were then designed for the amplification and sequencing of the 15 intergenic regions from genomic DNA of all 8 aye-aye individuals. All primers used in this study are provided in supplementary table 1. In addition, we sequenced a fragment that, based on BLAST analysis, is orthologous to an intergenic region on the human, chimpanzee (Pan troglodytes), rhesus macaque (Macaca mulatta), and dog (Canis familiaris) X-chromosome. The pattern of single nucleotide polymorphism (SNP) variation for this region was consistent with an X-chromosome location also in aye-aye, based on finding heterozygous sites in female but not in male individuals. All nucleotide sequences from this study have been deposited in GenBank under the accession numbers EF667150-EF667293.

Opsin Genes Amplification and Sequencing

A ~4.5 kb nucleotide sequence from the lesser galago (Galago senegalensis) autosomal S-opsin gene (OPN1SW) including all five exons (Kawamura and Kubotera 2004) was obtained from GenBank (accession number AB111465). This sequence was used to design PCR primers for amplification and sequencing of OPN1SW from one aye-aye with TripleMaster Taq polymerase (Eppendorf, Westbury, NY). Aye-aye-specific primers were then designed for amplification and sequencing of OPN1SW from the other 7 individuals. Based on functional and molecular comparisons, it is possible to estimate opsin wavelength absorption spectra from their inferred amino acid sequences (Neitz et al. 1991; Yokoyama and Radlwimmer 1999; Yokoyama and Radlwimmer 2001). Although there is no experimental evidence, some have speculated from the inferred amino acid sequence alone that the aye-aye S-opsin gene may encode an ultraviolet-absorbing pigment, rather than a blue-absorbing pigment as in other primates (Hunt et al. 2007). The literature distinguishes between these two pigments with different terminology; however, for simplicity, here we retain the terminology “S-opsin” throughout as we are referring to the gene and not to the potential wavelength absorbance of the pigment. Based on the inferred amino acid sequence of the X-linked opsin gene, Tan et al. (1999) estimated that the aye-aye has an M-opsin gene (OPN1MW). For this gene, we designed primers from human, mouse, and rat homologous nucleotide sequences to amplify and sequence the region including exons 3–5 from one aye-aye, followed by the design of aye-aye-specific primers for amplification and sequencing of the OPN1MW gene from the other 7 individuals.

Statistics and Coalescent Simulations

All nucleotide sequences were aligned and analyzed using Sequencher v. 4.2 (Gene Codes, Ann Arbor, MI). Estimates of the nucleotide diversity population parameter 0 for each of the 15 intergenic regions as well as the two opsin genes were calculated with two statistics: a sample-weighted estimate based on the number of single nucleotide polymorphisms (SNPs) per site (θW; Watterson 1975) and an estimate based on the average number of pairwise differences among sequences per site (θs). The overall average θW and θs estimates for the 15 intergenic regions are weighted by the length of each of the fragments. We also computed Tajima’s (1989) D statistic (which compares the values θW and θs), to examine our regions for skews in the SNP frequency distribution, where significant positive and negative values reflect excesses of high and low frequency SNPs, respectively.

Because we have no prior expectation for what “neutral” SNP frequency distributions embody, these values for the 15 intergenic regions served as a proxy from the aye-aye genome. We performed coalescent simulations using the observed levels of variation at the intergenic regions, both as θW and θs, as expected neutral values and generated 10,000 genealogies to determine how often observed levels of nucleotide diversity at the opsin genes fit these simulated distributions under a standard model of neutrality. Simulations run for the autosomal S-opsin gene were based on 16 chromosomes, whereas those run for the X-linked M-opsin gene were based on 13 chromosomes (5 females and 3 males). The simulations for the X-linked M-opsin gene were modeled on the premise that X-chromosomes have an expected effective population size 3/4 that of the autosomes (because males have one copy), which results in a conservative test under a model of neutrality. Coalescent simulations, Tajima’s (1989) D, and θW and θs, estimates were calculated using the DnaSP v. 4.1 software package (Rozas et al. 2003).
Table 1

<table>
<thead>
<tr>
<th>Region a</th>
<th>bp b</th>
<th>S c</th>
<th>ω 0 (%) d</th>
<th>ω 1 (%) d</th>
<th>Tajima’s D e</th>
</tr>
</thead>
<tbody>
<tr>
<td>chr1p33</td>
<td>1,000</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>N/A</td>
</tr>
<tr>
<td>chr6q15</td>
<td>1,076</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>N/A</td>
</tr>
<tr>
<td>ENr211</td>
<td>2,262</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>N/A</td>
</tr>
<tr>
<td>ENr111</td>
<td>2,242</td>
<td>2</td>
<td>0.027</td>
<td>0.023</td>
<td>−0.368</td>
</tr>
<tr>
<td>ENr121</td>
<td>1,940</td>
<td>3</td>
<td>0.047</td>
<td>0.034</td>
<td>−0.783</td>
</tr>
<tr>
<td>chr9q31.2</td>
<td>1,273</td>
<td>2</td>
<td>0.047</td>
<td>0.050</td>
<td>0.146</td>
</tr>
<tr>
<td>chr9q22.3</td>
<td>1,165</td>
<td>2</td>
<td>0.052</td>
<td>0.054</td>
<td>0.112</td>
</tr>
<tr>
<td>chr9q22.1</td>
<td>1,974</td>
<td>4</td>
<td>0.061</td>
<td>0.061</td>
<td>−0.004</td>
</tr>
<tr>
<td>chr1q12.2</td>
<td>1,032</td>
<td>2</td>
<td>0.058</td>
<td>0.067</td>
<td>0.375</td>
</tr>
<tr>
<td>ENr321</td>
<td>2,392</td>
<td>6</td>
<td>0.076</td>
<td>0.096</td>
<td>0.923</td>
</tr>
<tr>
<td>ENr123</td>
<td>1,535</td>
<td>5</td>
<td>0.098</td>
<td>0.106</td>
<td>0.263</td>
</tr>
<tr>
<td>Tar121</td>
<td>2,296</td>
<td>9</td>
<td>0.118</td>
<td>0.113</td>
<td>−0.160</td>
</tr>
<tr>
<td>chr1p12</td>
<td>1,528</td>
<td>7</td>
<td>0.130</td>
<td>0.161</td>
<td>0.585</td>
</tr>
<tr>
<td>ENr313</td>
<td>2,044</td>
<td>8</td>
<td>0.118</td>
<td>0.177</td>
<td>1.805</td>
</tr>
<tr>
<td>chr3q26.32</td>
<td>1,890</td>
<td>13</td>
<td>0.207</td>
<td>0.205</td>
<td>0.042</td>
</tr>
<tr>
<td>Total</td>
<td>25,649</td>
<td>63</td>
<td>0.074</td>
<td>0.081</td>
<td>0.402</td>
</tr>
</tbody>
</table>

a Names based on orthology to ENCODE regions or the corresponding chromosome position in humans (see Materials and Methods).
b Number of independent intergenic regions.
c Number of SNPs.
d Nucleotide diversity based on the number of SNPs (Watterson 1975).
e Nucleotide diversity based on the average pairwise sequence divergence.

# Results

## Aye-aye Population and Comparative Genetics

Our analysis of nucleotide sequence data from 15 intergenic regions ranging from 1-2 kb each (>25 kb in total) from each of 8 aye-aye individuals (16 chromosomes) found wide variance in estimates of SNP diversity (table 1), with three regions containing no polymorphism and one region containing 13 SNPs. Using Tajima’s (1989) test, we observed similar patterns for SNPs in magnitude and frequency, both for the overall dataset of >25 kb (ω 0 = 0.074% and ω 1 = 0.081%, respectively) and for the 15 regions independently (table 1). Thus, it appears that variation within our intergenic regions is an appropriate proxy for neutral variation in the aye-aye genome.

Although we collected nucleotide sequence for these intergenic regions primarily to obtain estimates of neutral levels of variation for comparison to aye-aye opsin genes, this sample also represents the first time that population-level intergenic region nucleotide sequence diversity data have been available for a non-hominoid primate species. In comparing nucleotide diversity across different primate genomes, including humans, we may determine how historical population sizes differed from today. This is particularly interesting for the aye-aye given its endangered status. Estimates for human, chimpanzee, bonobo (Pan paniscus), gorilla (Gorilla gorilla), and orangutan (Pongo pygmaeus) shown in table 2 were obtained using a similar approach to ours (i.e., multiple, independent, intergenic regions; Yu et al. 2002; Yu et al. 2003; Yu et al. 2004;Voight et al. 2005; Fischer et al. 2006). As is true of our estimate in aye-ayes, nucleotide diversity also varies substantially across intergenic regions in these other primates, including humans, which in part could simply reflect stochastic variation in evolutionary processes such as genetic drift (e.g., Hellmann et al. 2005). From our sample, estimates of aye-aye genetic diversity are similar to those of bonobos and humans, while estimates for chimpanzees, gorillas, and especially orangutans, are considerably greater. This range of estimates likely reflects differences in population sizes among these primates. Comparisons with additional genomic regions and species will help to determine whether the aye-aye population size borders on the low side for primates, which has implications for conservation strategies, or if low nucleotide diversity is characteristic of strepsirhine primates overall.

## Testing Opsi n Gene Evolutionary Hypotheses

For each of the 8 aye-aye individuals, we sequenced exon and intron regions from both the autosomal S-opsin gene and the X-linked M-opsin gene. Table 2 provides a comparison of nucleotide diversity across functional regions at each of the opsin genes and with our 15 putatively neutral intergenic regions. For the opsin genes, introns and synonymous sites within exons were combined into a “silent” class. Although such sites are not completely impervious to natural selection (e.g., Pagani et al. 2005; Parmley et al. 2006), they best reflect our expectation for neutral evolution compared to changes that alter amino acids and protein sequence (e.g., nonsynonymous sites).

Using the coalescent simulations discussed above, we find that the pattern of variation at silent sites in the S-opsin gene (ω 1 = 0.132%) is not significantly different than that expected based on the intergenic region variation (P = 0.88), and thus, appears to reflect neutral variation. In contrast, we identified only one singleton SNP among 784 S-opsin nonsynonymous sites (ω 1 = 0.016%). Although this estimate represents only a singleton variant, we cannot reject the null hypothesis that this observation is consistent with neutrality using our coalescent modeling because there are only 784 nonsynonymous sites (P = 0.10). In fact, due to this lack of statistical power, the observation of zero nonsynonymous SNPs would still not reject neutrality in this

### Table 2

<table>
<thead>
<tr>
<th>Species a</th>
<th>N b</th>
<th>Regions c</th>
<th>Total bp</th>
<th>ω 0 (%) d</th>
<th>ω 1 (%) d</th>
</tr>
</thead>
<tbody>
<tr>
<td>Aye-aye</td>
<td>16</td>
<td>15</td>
<td>25,649</td>
<td>0.07</td>
<td>0.08</td>
</tr>
<tr>
<td>Orangutan</td>
<td>32</td>
<td>19</td>
<td>16,001</td>
<td>0.35</td>
<td>0.36</td>
</tr>
<tr>
<td>Gorilla</td>
<td>30</td>
<td>49</td>
<td>23,056</td>
<td>0.15</td>
<td>0.16</td>
</tr>
<tr>
<td>Bonobo</td>
<td>13</td>
<td>50</td>
<td>23,500</td>
<td>0.08</td>
<td>0.08</td>
</tr>
<tr>
<td>Chimpanzee</td>
<td>34</td>
<td>50</td>
<td>23,500</td>
<td>0.19</td>
<td>0.13</td>
</tr>
<tr>
<td>Human</td>
<td>60</td>
<td>50</td>
<td>118,259</td>
<td>0.12</td>
<td>0.09</td>
</tr>
</tbody>
</table>

a Data compiled from previous studies for orangutan (Fischer et al. 2006), gorilla (Yu et al. 2004; Fischer et al. 2006), bonobo and chimpanzee (Yu et al. 2003; Fischer et al. 2006), and human (Yu et al. 2002; Voight et al. 2005). The orangutan and chimpanzee samples included individuals from multiple subspecies.
b Number of chromosomes.
c Number of independent intergenic regions.
d Nucleotide diversity based on the number of SNPs (Watterson 1975).
e Nucleotide diversity based on the average pairwise sequence divergence.

# References

Table 3
Comparison of Aye-aye Opsin and Intergenic Region Nucleotide Diversity

<table>
<thead>
<tr>
<th>Region</th>
<th>Class</th>
<th>Total bp</th>
<th>$S$</th>
<th>$\theta_N$ (%)</th>
<th>$\theta_{ST}$ (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>S-opsin</td>
<td>Silent</td>
<td>2,112</td>
<td>6</td>
<td>0.086</td>
<td>0.132</td>
</tr>
<tr>
<td></td>
<td>Nonsynonymous</td>
<td>784</td>
<td>1</td>
<td>0.038</td>
<td>0.016</td>
</tr>
<tr>
<td>M-opsin</td>
<td>Silent</td>
<td>4,042</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>Nonsynonymous</td>
<td>434</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>15 autosomal</td>
<td>intergenic</td>
<td>25,649</td>
<td>63</td>
<td>0.074</td>
<td>0.081</td>
</tr>
<tr>
<td>X-linked</td>
<td>intergenic</td>
<td>1,166</td>
<td>5</td>
<td>0.130</td>
<td>0.092</td>
</tr>
</tbody>
</table>

a For the autosomal S-opsin gene and autosomal intergenic regions, our sample of 8 individuals results in 16 chromosomes, whereas for the X-linked M-opsin gene and intergenic region our sample size is 13 chromosomes (5 females and 3 males).

b Opsin gene silent class includes intronic and synonymous coding region sites; untranslated regions (UTR) and splice sites were excluded from this analysis.

c Nucleotide diversity based on the number of SNPs (Watterson 1975).

d Nucleotide diversity based on the average pairwise sequence divergence.

e For purposes of direct comparison with autosomal regions, corrected $\theta_N$ and $\theta_{ST}$ values for the X-linked intergenic region (multiplied by 4/3) are 0.173% and 0.123%, respectively.

Discussion

Aye-ayes last shared a common ancestor with other extant lemurs at least 60 MYA and probably earlier (Tavare et al. 2002; Yoder and Yang 2004). Therefore, due to relatively high levels of nucleotide sequence divergence over this period of time, a between-species comparison of functional (e.g., nonsynonymous) and putatively neutral (e.g., synonymous) sites (i.e., $d_{s}/d_{s}$ test; Yang and Bielawski 2000) at opsin genes would only reveal long-term evolutionary changes in functional constraint. In contrast, in this study, we conducted the first within-species population analysis of the aye-aye S- and M-opsin genes in examining polymorphism frequency distributions that reflect recent evolutionary changes in functional constraint.

In testing our hypotheses, it was necessary to have an understanding of neutral levels of variation in the aye-aye genome. Therefore, we sequenced >25 kb from 15 intergenic regions in an aye-aye population sample; the first such data for a non-hominoid primate species. These data fit normal expectations under neutrality, with no significant skews in polymorphism frequency distributions toward rare or common alleles, which (in the absence of natural selection) would otherwise signify recent population expansion or bottlenecks, respectively (Tajima 1989).

In addition, for both autosomal and X-chromosome intergenic regions we found no evidence of excess homozygosity within individuals (e.g., skewed Hardy-Weinberg frequencies) or lower inter-allelic variability within individuals than among individuals, which may otherwise be expected if our sample was subject to inbreeding or an unusual mating structure. Together, these results suggest that an unusual population demographical history for our aye-ayes is unlikely to explain the low variation found at opsin gene functional sites.

Interestingly, we did not identify any variable sites in the X-linked M-opsin gene. This was also true for a few of the 15 intergenic regions; however, given that the size of the M-opsin gene is much larger at 4,476 bp, our coalescent simulations find that the complete absence of variation at this gene is highly unexpected under a model of neutral evolution ($P < 0.005$). One possible explanation for this significant deviation compared to the intergenic regions is that the aye-aye X-chromosome, because of its reduced effective population size (males have only one copy), exhibits far less diversity than autosomes. Although this was factored into our simulations, we empirically investigated this possibility by sequencing in our aye-aye samples a 1,166-bp fragment that aligns to an intergenic region on the human, chimpanzee, rhesus macaque, and dog X-chromosome.

Interestingly, this aye-aye intergenic region exhibits one of the highest nucleotide diversities compared to the 15 intergenic regions from autosomes (table 3). Therefore, this stark contrast suggests that the lack of variation at the M-opsin gene is not a reflection of low levels of X-chromosome neutral variation in general. This lack of variation could reflect purifying selection or a recent selective sweep or both. While we cannot distinguish among these hypotheses without extended-haplotype sampling outside this gene region, both scenarios are inconsistent with relaxed functional constraint and neutrality.
behavioral and ecological stability (i.e., inconsistent with the possibility of a very recent shift to nocturnality) and, combined with our molecular population genetic analyses, functional constraint for both the aye-aye S- and M-opsin genes under a nocturnal activity pattern over an extended period of evolutionary time. Although without further behavioral and experimental studies (e.g., Jacobs et al. 2002) we cannot exclude the possibility that both opsins are not expressed and purifying selection has maintained intact S- and M-opsin genes in the aye-aye for benefits other than color vision, the most likely explanation is that dichromacy is advantageous in some nocturnal primates. It is unclear what the advantage(s) of color vision in nocturnal primates may be, but possibilities include more effective detection and avoidance of diurnal predators that can disturb nocturnal animals from their sleeping sites (e.g., see Bearder et al. 2001), enhanced vision during any (possibly seasonal) dusk/dawn activity, or the ability to perceive color while foraging under moonlight conditions. With regard to night-time color vision perception, previous studies have shown that nocturnal geckos and hawkmoths, two widely separate taxa that each have color vision, are able to discriminate colors in light levels that approximate conditions of dim moonlight and starlight, respectively (Kelber et al. 2002; Roth and Kelber 2004; Kelber and Roth 2006).

If dichromacy is advantageous and has been maintained by natural selection in some nocturnal primates, then issues of considerable interest include why the same was not true for other nocturnal primates such as dwarf lemurs, lorises and galagos, and owl monkeys, all of which have monochromacy as a result of S-opsin gene degradation, and why functional loss of the S-opsin gene apparently occurred at different times among these lineages as suggested by considerable variation in the extent of S-opsin degradation (fig. 1; Jacobs et al. 1993, 1996; Kawamura and Kubotera 2004; Tan et al. 2005). Detailed behavioral and ecological comparisons between dichromatic and monochromatic nocturnal primate taxa may provide considerable insight into these issues. This would contribute greatly to our understanding of the evolution of primate nocturnal behavior in general—especially since primates evolved from a common ancestor that itself was likely dichromatic and is commonly suggested to have been nocturnal (e.g., Martin 1990; Heesey and Ross 2001; Martin and Ross 2005; Bearder et al. 2006; Ross and Kirk 2007). A particularly informative comparison may be between the potentially dichromatic mouse lemurs (which have intact S- and L-opsin genes) and monochromatic dwarf lemurs (with S-opsin gene degradation; Tan et al. 2005), because these taxa belong to the same primate family (Cheirogaleidae), are often sympatric, and overlap in many aspects of behavior and diet (e.g., Hladik 1979; Atsalis 1999). Dichromacy may also be relatively common for nocturnal non-primate mammals (Ahnelt and Kolb 2000; Bowmaker and Hunt 2006); for example, the insectivorous nocturnal microbat Myotis velifer has intact copies of both S- and L-opsin genes (Wang et al. 2004). Establishing whether specific nocturnal mammals have monochromatic or dichromatic vision and considering this in the context of their behavioral ecology will be an important area of future investigation.

Conclusion

We have used population genetic analyses to show that dichromatic vision likely remains beneficial in at least one nocturnal primate species, the aye-aye of Madagascar. Future behavioral studies of aye-ayes in their natural habitat could provide insight into the ecological contexts under which color vision might be advantageous. Unfortunately, this unique primate is endangered and such opportunities may be limited. The surprising findings of our study further emphasize the importance and incredible diversity of visual systems in the evolution of primates. For example, among extant nocturnal primates there may be important, and potentially subtle, differences that have influenced variable selection pressures on the S-opsin gene. Additionally, once pseudogenization has occurred and multiple disrupting mutations have accumulated, a reversal to restored function is unlikely, and this may now constrain the ecological niche of monochromatic nocturnal lineages, including dwarf lemurs, lorises and galagos, and owl monkeys. Our study highlights the advantages of genome-wide population analyses for providing insights into the evolutionary history of primates and other organisms.

Supplementary Material

Supplementary table 1 is available at Molecular Biology and Evolution online (http://www.mbe.oxfordjournals.org/).

Acknowledgments

We thank N.J. Dominy for comments on an earlier draft of the manuscript; R.Y. Tito for technical assistance; W.-H. Li, Y. Tan, A. Di Rienzo for sharing published sequence data, and S. Combes, J. Pastorini, A.C. Stone, the Duke Lemur Center (Durham, North Carolina), and the Jersey Zoo (United Kingdom) for the aye-aye samples. This work was supported by funds from the Center for Evolutionary Functional Genomics in The Biodesign Institute and the School of Life Sciences at Arizona State University (to B.C.V).

Literature Cited

Bearder SK, Nekaris KAI, Buzzell CA. 2001. Dangers in the night: Are some nocturnal primates afraid of the dark?. In: Miller LE, editor. Eat or be eaten: Predator sensitive foraging...


