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ORIGINAL ARTICLE

Less is more: lemurs (*Eulemur* spp.) may benefit from loss of trichromatic vision

Rachel L. Jacobs[1] · Carrie C. Veilleux[2] · Edward E. Louis Jr[3] · James P. Herrera[4] · Chihiro Hiramatsu[5,6] · David C. Frankel[1] · Mitchell T. Irwin[7] · Amanda D. Melin[2,8] · Brenda J. Bradley[1]

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Abstract

Vertebrate color vision is an ideal system for studying the gains and losses of genetic variation across lineages and impacts on behavior. Among placental mammals, trichromatic vision is unique to primates and is argued to be adaptive for foraging on reddish food. However, trichromacy is variably present in lemurs, including species within the cathemeral genus *Eulemur*, due to inter- and intra-specific variation in X-linked opsin genes. Although this variation could result from genetic drift, it could also reflect ecological adaptation. To understand ecological contributions to color vision variation, we examined cone opsin genes of 11 *Eulemur* species. We found that only *E. flavifrons* and *E. macaco* have polymorphic trichromacy. Most dichromatic species have an "M" (green-shifted) opsin; uniquely, one species (*E. rubriventer*) has dichromacy based on an "L" (red-shifted) opsin. This latter result appears to represent loss of polymorphic trichromacy from a dichromatic (M opsin) or polymorphic *Eulemur* ancestor. To address potential ecological explanations for opsin variation, we studied the dietary behavior of wild *E. rubriventer* and collected reflectance spectra from plant species consumed. Visual models suggest that trichromacy should provide an advantage for detecting reddish foods; however, luminance contrasts were greatest for dichromats with the L opsin. As *E. rubriventer* are often active in low-light rainforest conditions, luminance cues may be relatively important, which could favor the L opsin, while also leading to relaxed selection on, or selection against, trichromacy. The presence of different opsin alleles across *Eulemur* species could represent adaptations related to diet, activity pattern, or habitat.

Significance statement

Loss of genetic variation, often thought to be maladaptive, can occur through natural selection. Among primates, some species have trichromatic color vision, the ability to distinguish reddish and greenish hues; others are red-green colorblind (dichromatic).

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Amanda D. Melin and Brenda J. Bradley joint senior authors

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✉ Rachel L. Jacobs
rlynjacobs@gmail.com

[1] Center for the Advanced Study of Human Paleobiology, Department of Anthropology, The George Washington University, Washington, DC 20052, USA

[2] Department of Anthropology and Archaeology, University of Calgary, Calgary, AB, Canada

[3] Conservation Genetics Department, Omaha's Henry Doorly Zoo and Aquarium, Omaha, NE 68107, USA

[4] Department of Evolutionary Anthropology, Duke University, Durham, NC 27708, USA

[5] Department of Human Science, Faculty of Design, Kyushu University, 4-9-1 Shiobaru, Minamiku, Fukuoka 815-8540, Japan

[6] Physiological Anthropology Research Center, Kyushu University, 4-9-1 Shiobaru, Minamiku, Fukuoka 815-8540, Japan

[7] Department of Anthropology, Northern Illinois University, DeKalb, IL 60115, USA

[8] Department of Medical Genetics and Alberta Children's Hospital Research Institute, University of Calgary, Calgary, AB, Canada

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We examined adaptive explanations for color vision differences by studying cone opsin genes and behavior in wild lemurs (*Eulemur*)—a genus that is active both day and night. We found that color vision is variable in *Eulemur* species, and full trichromatic vision was likely lost in at least one lineage. Foraging ecology of dichromatic *Eulemur rubriventer* indicates that trichromatic vision should be advantageous for foraging on reddish foods, but brightness cues are more salient to this species' vision. We suggest brightness may be more important than color to this species, particularly at night, and loss of trichromacy could be adaptive in some lemurs.

Keywords Adaptation · Diversity · Luminance · Opsin · Polymorphic trichromacy · Sensory ecology

Introduction

Loss of genetic variation in wild populations is often assumed to be a maladaptive result of genetic drift or inbreeding (Frankham 2005). Indeed, conservation biologists frequently view such losses as an indicator of declining population size and viability (Frankham 1996; Lacy 1997; Reed and Frankham 2003). However, genetic variation can be influenced by multiple evolutionary mechanisms, sometimes pushing allele frequencies in the same direction. For example, adaptive mechanisms like directional selection can also drive the loss of allelic variation (Futuyma 1998), and in a given context "less is more" for some loci. That is, greater allelic variation is not necessarily advantageous and, in some cases, might even be disadvantageous (Futuyma 1998), favoring fewer alleles at a given locus.

The color vision system of vertebrates provides a notable case study in the repeated gain and loss of genetic variation across lineages (e.g., Hunt et al. 1998; Rennison et al. 2012; Borges et al. 2015). Of particular interest, many primates, including nearly all New World monkeys (platyrrhines) and some day-active lemurs, show intra-specific variation in color vision; they have multiple alleles at the single X-linked opsin gene, resulting in "polymorphic trichromacy" (Tan and Li 1999; reviews in Surridge et al. 2003; Veilleux and Bolnick 2009; Kawamura et al. 2012; Jacobs et al. 2017). This pattern differs from other trichromatic primates (e.g., Old World monkeys, apes, and humans), which have two different X-linked opsin genes, and as such, virtually, all individuals have trichromatic color vision (Dulai et al. 1999; Jacobs and Deegan 1999; Nathans 1999; Fig. S1). In species with polymorphic trichromacy, heterozygous females have trichromatic color vision, while homozygous females and all males are red-green colorblind (i.e., dichromatic; Jacobs and Neitz 1987; Williams et al. 1992; Jacobs et al. 1993; Fig. S1).

Polymorphic color vision in primate populations is often cited as an example of adaptive molecular evolution favoring and maintaining allelic diversity (Surridge et al. 2003; Kawamura and Melin 2017). This system, with its well-established genotype-phenotype link (e.g., Bradley and Lawler 2011), provides unique opportunities to address evolutionary questions about how variation is maintained and lost in populations. Accordingly, there has been a large amount of research, particularly in New World monkeys, aimed at identifying the selective mechanisms resulting in balancing selection (e.g., heterozygous advantage, niche differentiation, group benefit of association; Melin et al. 2007, 2008, 2017a; Smith et al. 2012; Veilleux et al. 2016). Although the mechanisms maintaining variation might differ across species and populations, it is reasonable to hypothesize that trichromatic color vision should provide an advantage, or else variation would likely be lost due to allelic drift (Futuyma 1998), and studies of allelic diversity support this argument (Hiwatashi et al. 2010). Until recently, trichromatic advantage remained largely in the realm of theory (e.g., Osorio et al. 2004) with limited evidence for advantages in wild populations. Recent research on New World monkeys, however, has found that trichromatic color vision provides foraging advantages through higher intake rates for trichromats when feeding on yellowish-reddish fruit compared to dichromats (Melin et al. 2017a; but see e.g., Vogel et al. 2007), and more frequent visits to flower patches by trichromatic individuals (Hogan et al. 2018). There is also some evidence that trichromacy provides foraging advantages in polymorphic lemur species (Veilleux et al. 2016). There may be additional advantages to trichromatic individuals related to detecting young leaves (Dominy and Lucas 2001; Melin et al. 2017b), predators (Pessoa et al. 2014), and social signals of conspecifics (Hiramatsu et al. 2017). Taken together, these results support the hypothesis that trichromatic color vision is adaptive and should be favored by natural selection.

What is puzzling then is the potential loss of polymorphic trichromatic color vision in some primates, notably some *Eulemur* species (Jacobs and Bradley 2016). Members of this lineage are cathemeral (active day and night) and eat a large amount of fruit. Although other closely related lemurs with similar biology have polymorphic trichromacy (e.g., genus *Varecia*; Tan and Li 1999; Jacobs and Deegan 2003), it has so far been found in a single species of *Eulemur*, *E. flavifrons* (Veilleux and Bolnick 2009); other congeners (*E. fulvus*, *E. collaris*, *E. mongoz*, *E. rubriventer*) appear to be dichromatic (Tan and Li 1999; Leonhardt et al. 2009; Jacobs and Bradley 2016; Valenta et al. 2016). Interestingly, different X-linked opsin alleles appear to be fixed in different dichromatic

species. While the polymorphic *E. flavifrons* has two X-linked opsin alleles (one "M" (green-shifted) opsin with peak spectral sensitivity [λ_{max}] ~ 543 nm and one "L" (red-shifted) opsin with λ_{max} ~ 558 nm; Veilleux and Bolnick 2009; Fig. S1), among the dichromatic congeners, three species (*E. fulvus*, *E. collaris*, *E. mongoz*) exhibit the single M opsin, while one (*E. rubriventer*) has the L opsin (Tan and Li 1999; Leonhardt et al. 2009; Jacobs and Bradley 2016; Valenta et al. 2016). This distribution of M and L opsins among *Eulemur* species suggests that polymorphic trichromacy may have been the ancestral *Eulemur* condition and was subsequently lost in some species or populations (Jacobs and Bradley 2016), a scenario similarly proposed for another dichromatic primate clade, the nocturnal tarsiers (Melin et al. 2013). Importantly, even given a dichromatic *Eulemur* ancestry (either M or L opsin), polymorphic trichromacy would have been lost in one or more species, as a polymorphic condition, even if brief, must occur to transition between a M and L opsin.

Given that trichromacy appears to be adaptive for foraging on fruit (e.g., Melin et al. 2017a), loss of this variation in *Eulemur* seems surprising (Jacobs and Bradley 2016), because those species are day-active and largely frugivorous (Mittermeier et al. 2010). Allelic loss could be a maladaptive or neutral result of drift (e.g., genetic bottlenecks), but genetic evidence for this mechanism is equivocal (Jacobs and Bradley 2016). We therefore examined whether loss of trichromacy in *Eulemur* could be adaptive. For example, recent research suggests that fruit colors in a Malagasy forest (Ranomafana National Park) may be less visually conspicuous in the red-green color channel compared to fruit colors in other forests in Africa (Kibale National Park, Uganda; Nevo et al. 2018). Moreover, although potentially variable across sites (Bollen et al. 2005), those fruits consumed by some lemurs (including some *Eulemur* species) in Madagascar may be primarily "dull" in coloration (i.e., green and brown; Dew and Wright 1998; Birkinshaw 2001; Bollen et al. 2005). If trichromacy does not provide an advantage in detecting food items consumed by some lemur species/populations, then we might expect selection favoring trichromatic color vision to be relaxed, which could lead to loss of polymorphic trichromacy. Alternatively (or additionally), there could be selection favoring particular opsin variants in dichromatic taxa. Indeed, using color modeling techniques, it has been suggested that L-based dichromacy is superior to M-based dichromacy for detecting chromatic cues of food items consumed by nocturnal woolly lemurs, genus *Avahi*, in Ranomafana National Park (Veilleux et al. 2014).

Eulemur, therefore, represents an ideal lineage to explore evolutionary mechanisms that could result in loss of variation. The aims of this study were threefold. First, we examined genetic variation across the genus *Eulemur* (11 species and 22 populations) to better characterize the distribution of M and L opsins across populations and species. Second, we explored patterns of potential allele loss in the genus *Eulemur* by estimating the ancestral color vision state. Finally, we assessed potential ecological pressures related to the visual ecology of dichromatic lemurs, using red-bellied lemurs in Ranomafana National Park as a case study. This population of *E. rubriventer* is monomorphic for the L opsin (Jacobs and Bradley 2016), and therefore seems to differ from other *Eulemur* species that are either polymorphic or monomorphic for the M opsin. If trichromacy was lost in this population due to relaxed selection pressures, we predict that the food items consumed will not be more salient to a trichromat compared to a dichromat (Sumner and Mollon 2000; Regan et al. 2001; Hiramatsu et al. 2008). If fixation of the L opsin might represent selection favoring the L opsin over the M opsin during foraging, we predict that the color of food items consumed by *E. rubriventer* will be more salient to dichromats with the L opsin compared to dichromats with the M opsin. To test these predictions, we measured reflectance spectra of *E. rubriventer* food items consumed during behavioral observations and then modeled food conspicuity to different color vision systems.

Methods

Study species

Eulemur species are medium-sized frugivorous lemurs that occur throughout Madagascar's varied forest habitats, including the western dry deciduous forests and the eastern rainforests, but they do not occur in the southern spiny forests (Mittermeier et al. 2010). *Eulemur* species are cathemeral, and species vary in the degree of diurnal and nocturnal activity (Donati et al. 2016). In this study, we examined 11 of the 12 recognized *Eulemur* species in Madagascar (Fig. 1). We generated opsin sequence data for: *E. coronatus*, *E. flavifrons*, *E. macaco*, *E. rubriventer*, and members of the so-called brown lemur complex (Markolf et al. 2013), including *E. albifrons*, *E. cinereiceps*, *E. collaris*, *E. fulvus*, *E. rufifrons*, and *E. sanfordi*. Opsin data were already available for captive *E. mongoz* (Tan and Li 1999). Thus, we examined the entire genus less one species, *E. rufus* (considered to be part of the "brown lemur complex").

X-linked opsin variation in *Eulemur*

Sample collection and DNA extraction

Blood/tissue or fecal samples were obtained from 142 individuals across 22 wild populations in Madagascar as part of separate research projects (Table 1; Fig. 1). Blood/tissue samples comprised the majority ($N = 138$ individuals from 21 populations), and sample collection methods have been

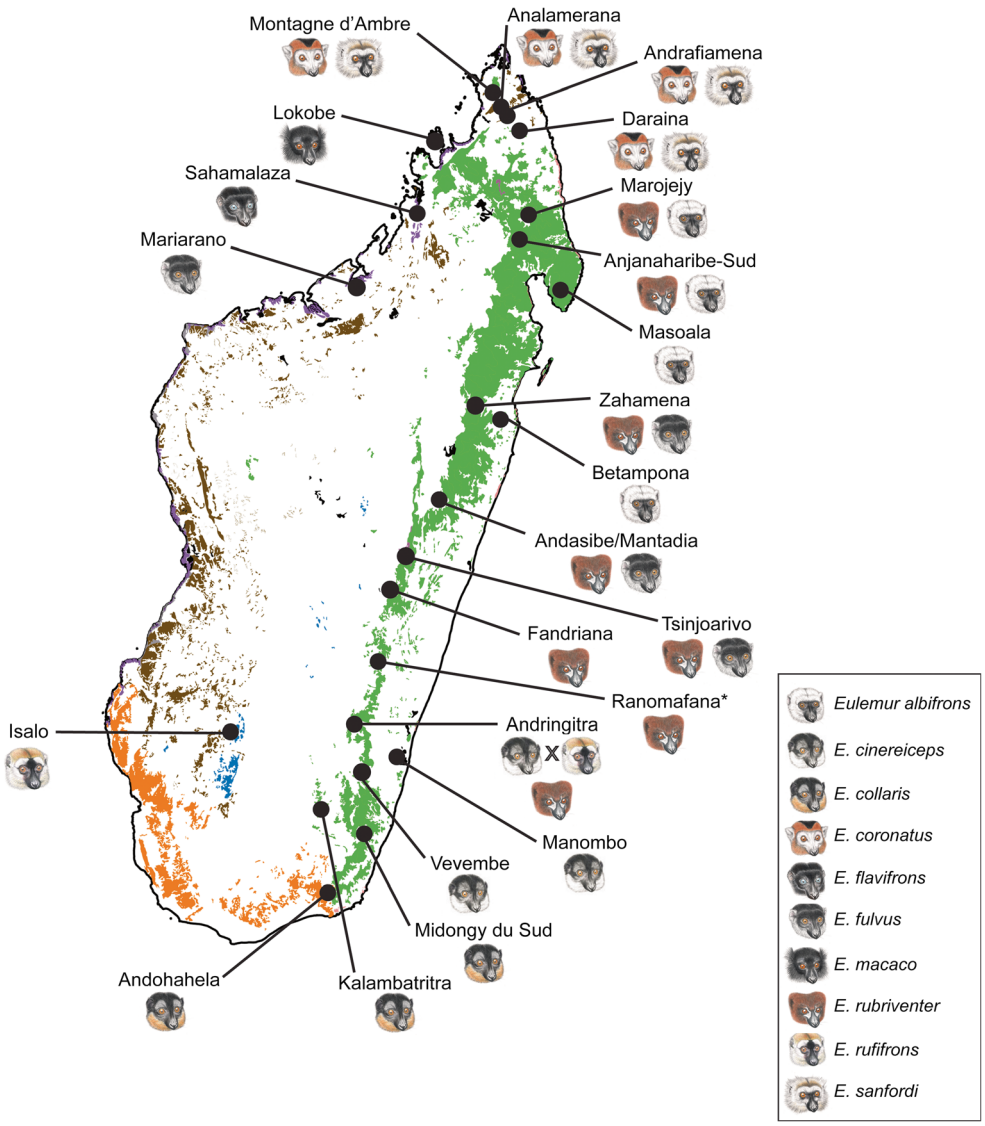

Fig. 1 Ten species of *Eulemur* were sampled across 22 sites in Madagascar. *Identifies Ranomafana National Park where foraging data were collected on red-bellied lemurs (*E. rubriventer*). The "X" under Andringitra indicates a hybrid population (*E. cinereiceps* x *E. rufifrons*). Illustrations copyright 2015 Stephen D. Nash/IUCN SSC Primate Specialist Group. Used with permission. Map from Du Puy and Moat (1996)

previously described in detail (Brenneman et al. 2012). For those samples, we extracted genomic DNA from blood/tissue using standard phenol-chloroform-isoamyl extraction protocols (Sambrook et al. 1989). Fecal samples were collected from four individuals at the Tsinjoarivo site. Sample collection/preservation methods followed the two-step protocol (ethanol-silica gel beads; Nsubuga et al. 2004), and DNA extraction followed Jacobs and Bradley (2016). Negative controls were included in extractions and all downstream procedures. All genetic analyses were conducted at the George Washington University Primate Genomics Lab and the Conservation Genetics Laboratory at Omaha's Henry Doorly Zoo and Aquarium.

Opsin genotyping

In primates, spectral differences among medium-to-long wavelength sensitive opsins result primarily from amino acid changes from single-nucleotide polymorphisms (SNP) at three sites in the X-linked opsin gene (180 in exon 3; 277 and 285 in exon 5) (Neitz et al. 1991; Hiramatsu et al. 2004). All three sites vary in some lemurs (Jacobs et al. 2017), but among *Eulemur*, only site 285 is known to vary, resulting in two opsin alleles: "M" (λ_{max} ~ 543 nm with a three-site combination of alanine, tyrosine, alanine—AYA—for 180, 277, and 285, respectively) and "L" (λ_{max} ~ 558 nm with a three-site combination of alanine, tyrosine, threonine—AYT) (Tan and Li 1999; Veilleux and Bolnick 2009). We targeted exons 3 and 5 to capture the three key functional sites.

We amplified exons 3 and 5 using quantitative PCR (Mic qPCR Cycler, Bio Molecular Systems; Rotor-Gene Q, Qiagen), which was immediately followed by high-resolution melt analysis (HRMA; Jacobs et al. 2016). We assigned genotypes for each exon based on the shape and temperature of the melt curve compared to positive controls of known genotypes (identified by Sanger sequencing). Each

Table 1 Samples of *Eulemur* individuals for which X-linked opsin genotypes were obtained

Species	Site	N_{males}, $N_{females}$	$N_{X\ chromosomes}$
Eulemur albifrons	Anjanaharibe-Sud	2,3	8
	Betampona	3,2	7
	Marojejy	3,2	7
	Masoala	1,0	1
		9,7	23
Eulemur cinereiceps	Andringitra (hybrids)	2,3	8
	Manombo	3,2	7
	Vevembe	3,2	7
		8,7	22
Eulemur collaris	Andohahela	2,3	8
	Kalambatritra	3,2	7
	Midongy du Sud	2,3	8
		7,8	23
Eulemur coronatus	Analamerana	3,2	7
	Andrafiamena	3,0	3
	Daraina	2,0	2
	Montagne d'Ambre	3,2	7
		11,4	19
Eulemur flavifrons	Sahamalaza	7,3	13
		7,3	13
Eulemur fulvus	Andasibe/Mantadia	4,2	8
	Mariarano	1,4	9
	Tsinjoarivo	3,0	3
	Zahamena	3,2	7
		8,8	24
Eulemur macaco	Lokobe	1,2	5
		1,2	**5**
Eulemur rubriventer	Andringitra	2,3	8
	Andasibe	2,3	8
	Anjanaharibe-Sud	1,1	3
	Fandriana	3,2	7
	Marojejy	2,3	8
	Tsinjoarivo	0,1	2
	Zahamena	1,0	1
		11,12	35
Eulemur rufifrons	Andringitra (hybrids)	3,2	7
	Isalo	2,3	8
		5,5	15
Eulemur sanfordi	Analamerana	1,4	9
	Andrafiamena	2,0	2
	Daraina	2,1	4
	Montagne d'Ambre	3,2	7
		8,7	22
	Total	78,64	206

sample was replicated in two independent reactions per exon (four reactions per exon for fecal samples). For a subset of individuals of each species ($N = 52$ for exon 3; $N = 79$ for exon 5) representing the full range of observed melt temperature variation (Jacobs et al. 2016), HRMA genotype calls were further confirmed via Sanger sequencing electropherograms of qPCR amplicons. In all cases, sequences matched HRMA-based calls.

Ancestral color vision estimations and allele loss

To estimate the evolutionary history of opsin variation in the genus *Eulemur*, we coded the color vision for 52 species of lemuriforms, lorisiforms, and tarsiiforms as follows: 1—a single M opsin ($\lambda_{max} \leq 543$ nm), 2—polymorphic (two or more alleles), and 3—a single L opsin ($\lambda_{max} \geq 558$ nm) (see Table S1 for data and references). We used the recent phylogeny from Herrera and Dávalos (2016) and added the following missing taxa, based on literature estimating their divergence times: *Avahi mooreorum* and *Tarsius bancanus* (Lei et al. 2008; Springer et al. 2012). We mapped the evolution of the three-state character with an ordered transition matrix, such that changing from a single M opsin to a single L opsin or vice versa first required a transition to the polymorphic state. This ordering reflects the dynamics of the visual system. Allelic variation must be present for changes in allele frequencies to occur. Accordingly, changing from a monomorphic M or L opsin genotype (i.e., one opsin allele fixed) to a different monomorphic M or L opsin genotype (i.e., different opsin allele fixed) would necessitate that at some point in the evolutionary history of the population, both opsin alleles were present (e.g., an opsin allele was introduced through mutation or migration), resulting in polymorphic trichromacy. We simulated trait evolution using stochastic character mapping (Huelsenbeck et al. 2003) implemented in the package *phytools* (Revell 2012) for the R statistical environment (R Core Team 2014). Briefly, stochastic character mapping traces the evolution of a discrete trait on a tree using a continuous-time Markov model. This method first calculates the conditional likelihood of character states at each node, then simulates the evolution of the trait along the branches of the tree in proportion to the probability of the trait changes. This approach has the advantage of being a stochastic model of trait evolution that allows more than one state change to occur along branches. The probabilities of state changes on branches are proportional to time; more changes are likely to occur on long branches compared to short branches. The states at ancestral nodes were summarized by averaging, weighted by their probabilities. We ran 10,000 simulations with the ordered transition matrix, estimating the ancestral state at the root under a flat prior probability of 1/3 for each state (*make.simmap* function in *phytools*). The analyses were run in two ways: (1) using symmetrical transition rates and (2) allowing transition rates between states to differ.

Foraging ecology of *Eulemur rubriventer*

Study site

We collected foraging data on the population of *E. rubriventer* in Ranomafana National Park (RNP), which is an area of 41,000 ha of montane rainforest in southeastern Madagascar (E47° 18′–47° 37′, S21° 02′–21° 25′; Wright 1992). This study population was previously identified to be monomorphic for the X-linked opsin gene, exhibiting only an L opsin (AYT, $\lambda_{max} \sim 558$ nm; Jacobs and Bradley 2016). Individuals were identifiable for behavioral data collection based on variation in their pelage coloration/patterns (Jacobs and Bradley 2016).

E. rubriventer food items

From the end of September 2012 through mid-May 2013, we collected foraging data on 3 groups of red-bellied lemurs from each of three localities within RNP ($N = 9$ groups; Table 2). It was not possible to record data blind because our study involved focal animals in the field, but behavioral data were collected from focal animals prior to completion of opsin genotyping. We followed groups from the time of group location through dusk until sunset when light levels precluded visual observation of animals. We rotated sites monthly (every 10 days) and attempted to follow each group 3 days/month (Table 2). The goal of the behavioral follows was to characterize the diet of this population (i.e., identify plants, plant parts, and plant part colors that are consumed) based on the foraging behavior of 36 individuals, and thus pseudoreplication was not an issue.

During group follows, we recorded all occurrences (i.e., "bouts") of foraging. We defined bouts as when at least one individual in the group entered a new tree to feed or forage or when foraging resumed in a tree after all individuals had stopped feeding for at least 10 min. During foraging bouts, we recorded the species of the food item consumed (using the local vernacular species names), along with the plant part consumed and the color of the plant part consumed when possible. We defined plant parts as ripe and unripe fruit when visual color changes of the fruit allowed identification (i.e., the use of ripeness category in this study does not refer to quantified mechanical properties of food items). For some fruit, multiple color changes occur during ripening (e.g., green to yellow to red), in which case we assigned a category of "mid-ripe" post hoc based on the color of fruit consumed. For plant species in which the fruit does not exhibit conspicuous color changes during ripening (or if this was unknown), we defined items broadly as "fruit." Additional plant parts included flower buds, flowers, leaf petioles, young leaves, mature leaves, galls, and mushrooms.

Reflectance data

We collected food items consumed by *E. rubriventer* from known feeding trees within 10 days when a study group had been observed feeding. Occasionally (~ 15%

Table 2 Red-bellied lemur study groups, compositions, and number of days followed during behavioral data collection. (AM, adult males; AF, adult females; IM, immature males; IF, immature females)

Group	Locality	N_{AM}	N_{AF}	N_{IM}	N_{IF}	$N_{Infants}$	$N_{Total\ individuals}$	N_{Days}
TK3	Talatakely	1 (1) [a]	1	1	1	0	4-2	18
TK4	Talatakely	1	1	0	2	0	4	14
TK5	Talatakely	1	1	0	1	0	3	20
VT3	Vatoharanana	1	1	0	0	1	3	16
VT5	Vatoharanana	1	1	1	1	0	4	12
VT7	Vatoharanana	1	1	1	1	0	4	14
VL1	Valohoaka	1	1	1	2	0	5	15
VL5	Valohoaka	1	1	0	1	0	3	18
VL9	Valohoaka	1	1	1	2	0	5	22
		10	9	5	11	1	36	149

[a] The group composition of TK3 changed during the course of the study, beginning with four individuals and ending with two individuals (an adult male and adult female). The change in group composition included replacement of the original adult male with a new male

of instances), when known feeding trees were devoid of fruit on collection days, we obtained food items from other trees of the same species within the site. We collected food items directly from trees using an extendable tree pruner when possible. For many trees, however, the height of the tree precluded direct collection, and, in such cases, we collected "fresh" samples (i.e., excluding any overripe or decaying fruit) from the ground (Dominy 2004). Once collected, we placed samples into a cooler with ice packs and returned to the research station and measured the spectral reflectance of food items within 14 h of sample collection (Dominy and Lucas 2004).

We measured reflectance spectra of samples using a USB2000+UV–VIS Miniature Fiber Optic Spectrometer (Ocean Optics, Dunedin, FL) under standard lighting conditions (PX-2 Pulsed Xenon Light Source; Ocean Optics). We recorded measurements relative to a diffuse reflectance standard (WS-1; Ocean Optics) using a reflection probe maintained at a fixed angle (45°) and distance (5 mm) from each sample using a probe holder (RPH-1; Ocean Optics). We frequently recalibrated the spectrometer during data collection to minimize drift. Depending on the size of the sample, we collected multiple (1–5) measurements. In addition to food samples, we collected mature leaves from plant species when possible and recorded one to three measurements for the upper and lower part of each leaf. Food items and leaves were represented by 1–10 (mean = 4) individual samples, and we calculated mean reflectance for each item for data analysis.

Visual modeling analyses

To address the question of whether different color vision phenotypes could provide a foraging advantage, we analyzed the chromaticities and chromatic and achromatic contrasts of food items and calculated visual conspicuousness in units of "Just Noticeable Difference" (JNDs) based on the visual system of *Eulemur*.

Chromaticity analyses We calculated chromaticity using the quantum catch of cone photoreceptors for a trichromatic *Eulemur*: S = 413 nm, M = 543 nm, L = 558 nm (Veilleux and Bolnick 2009; Carvalho et al. 2012). Our calculations followed Hiramatsu et al. (2008) and Valenta et al. (2013, 2016), in which the quantum catch (Q) of each cone photoreceptor i (i.e., S, M, and L) across 400–700 nm, which represents the visual spectrum of primates, was based on the following formula:

$$Q_i = \int_{400}^{700} R(\lambda) I(\lambda) S_i(\lambda) \mathrm{d}(\lambda)$$

In this formula, λ refers to wavelength, R is the reflectance spectrum of the item, I is the spectrum of the illumination, and S is the spectral sensitivity of the cone photoreceptor. We did not include effects of macular pigment on the pre-receptoral filter as this feature is lacking in lemurs. Rather, we calculated functions using methods for lemurs following Valenta et al. (2013, 2016) and included only the effects of the lens.

We used three illumination spectra in our analyses: "day," "dusk," and "moonlit night" (Fig. 2), as we observed red-bellied lemurs to be active and feeding during daylight and dusk (low sun angles 10° to below the horizon; Endler 1993) conditions. Additionally, we include "moonlit night" (unobstructed moonlight) spectra because, although we did not continue group follows throughout the night, *E. rubriventer* has been noted to have extensive nocturnal activity, with two peaks around

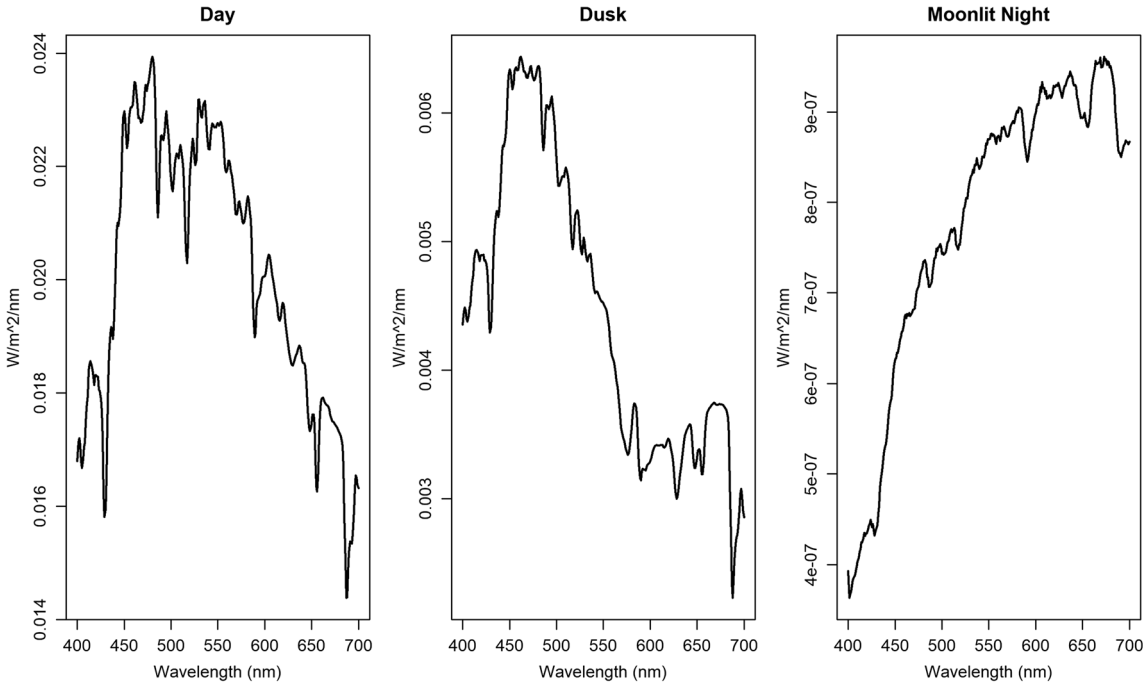

Fig. 2 Illumination spectra: "day" (1040 h), "dusk" (1720 h), "moonlit night" (1850 h). The former two were measured in Ranomafana National Park, and the latter was measured in Sabah, Malaysia. Data were converted to units of photon flux (μmol/m^2/s) for visual modeling analyses

1800–2200 h and 0100–0500 h (Overdorff 1996; Donati et al. 2016). Although some nocturnal conditions preclude the use of color vision, previous research indicates that light conditions at dusk and moonlit conditions at night are likely bright enough to enable color vision in primates (Roth et al. 2008; Melin et al. 2012). The illumination spectrum representing "day" was collected in RNP on April 29, 2013 under light shade (forest canopy) and overcast conditions at 1040 h. The illumination spectrum representing "dusk" was collected near sunset (1720 h) on the same day under open canopy and overcast conditions. These two illumination spectra were measured with down-welling light (probe directed upward) through a cosine corrector (CC-3-DA; Ocean Optics) directly attached to the USB2000+UV–VIS Miniature Fiber Optic Spectrometer. Finally, we used an unobstructed moonlight illumination spectrum taken in the tropical forests of Sabah, Malaysia (July 12, 2011, 1850 h; Melin et al. 2012) as representing a moonlit night. This spectrum was taken with a multichannel spectrometer with a highly sensitive photomultiplier detector and an integrating sphere to ensure a cosine angular response (OL-770VIS, Gooch & Housego, Orlando, FL). Spectra taken in Sabah agree well with irradiance spectra from other latitudes (Endler 1993; Johnsen et al. 2006), indicating that a similar pattern can be expected in Madagascar (cf. Pariente 1980). Irradiance spectra were converted from units of absolute irradiance (μW/m^2/nm) to units of photon flux (μmol/m^2/s) prior to calculating chromaticities and JNDs following Maia et al. (2013).

We calculated red-green chromaticity as the ratio of the quantum catch for L cones to L and M cones (i.e., L/(L+M); trichromats only). We calculated blue-yellow chromaticity as the ratio of quantum catch for S cones to L and/or M cones (i.e., S/(L+M) for trichromats; S/M or S/L for dichromats). Finally, we calculated luminance by dividing the quantum catch of L and/or M cones ((L+M) for trichromats; M or L alone for dichromats) by a hypothetical white surface that reflects 100% of the given illumination.

To determine if there is a potential foraging advantage for trichromatic *Eulemur*, we compared chromaticities and luminance of food items to those of mature leaves using non-parametric Mann-Whitney *U* tests. All statistical analyses here and in subsequent sections were performed in R Version 3.4.3 (R Core Team 2017) and were two-tailed with significance set at $p < 0.05$, followed with Bonferroni correction as appropriate.

Chromatic and luminance contrasts We calculated chromatic and luminance contrasts between each food item consumed and its respective mature leaf background (for upper and lower leaf backgrounds) for dichromatic *Eulemur*. In cases where data for mature leaves of the same species were unavailable, we used mean leaf background (using all mature leaves in the data set) in calculations.

Blue-yellow chromatic contrast was calculated as $|\ln\left(Q^f_{i_{L,M}}\right)-\ln\left(Q^b_{i_{L,M}}\right)|-|\ln\left(Q^f_S\right)-\ln\left(Q^b_S\right)|$. Luminance

contrast was calculated as $|\ln\left(Q^{f}_{i_{L,M}}\right)-\ln\left(Q^{b}_{i_{L,M}}\right)|$. Q is the quantum catch of L or M cones ($i_{L,\ M}$) for each dichromatic phenotype and S cones (S) for each food item (f) and mature leaf background (b). All calculations followed Hiramatsu et al. (2008) and were performed in Matlab.

To determine if relative chromatic or luminance contrast is greater for dichromats with the L opsin compared to dichromats with the M opsin, we used Wilcoxon signed-rank tests on food items against upper and lower leaf backgrounds for each illumination condition.

JND analyses JND calculations follow established methods (Osorio et al. 2004; Hiramatsu et al. 2008; Matsumoto et al. 2014; Valenta et al. 2016). We show the formula for trichromats below.

$$\mathrm{JND}=\sqrt{\frac{e_S{}^2(\Delta f_L-\Delta f_M)^2+e_M{}^2(\Delta f_L-\Delta f_S)^2+e_L{}^2(\Delta f_M-\Delta f_S)^2}{(e_Le_M)^2+(e_Le_S)^2+(e_Me_S)^2}}$$

where Δf_i is the difference in the quantum catch of receptor i between a food item and its upper or lower leaf background. The noise value (e_i) is set for each receptor type (i = S, M, L) by incorporating both the effect of quantum catch amount and cone proportion in the retina:

$$e_i=\sqrt{\frac{1}{f_iq_i}+\frac{w_i{}^2}{p_i}}$$

where q_i is the estimate of quantum flux (in terms of the number of photons) per cone cell per second in receptor i, w_i is the Weber fraction of receptor i, and p_i is the relative proportion of receptor i to the most abundant cone in the retina (Higham et al. 2010). The relative cone proportions follow Matsumoto et al. (2014) and Valenta et al. (2016). We adjusted maximum photon values for each light environment to represent the decreasing quantity of photons reaching the retina with lowering ambient light: $q_{L,\ M}=10^4$ ("day"), $q_{L,\ M}=10^3$ ("dusk"), $q_{L,\ M}=10^2$ ("moonlit night"). We set the value of quantum flux for S cones as $q_s=q_L/10$ to take account of their low sensitivity. We used Weber fraction values (w_i) of 0.08 for the S cones and 0.02 for both the M and the L cones. These values are close to psychophysical thresholds for humans (Wyszecki and Stiles 1982; Osorio et al. 2004). In the absence of species-specific data on relative cone proportions in the retinas, we set p_L, $p_M=1$, $p_s=0.1$ (Martin and Grunert 1999; Hiramatsu et al. 2008). JND values were calculated for each *Eulemur* color vision phenotype, and we assessed differences using Wilcoxon signed-rank tests.

Data availability Opsin sequence data generated during this study are available as electronic supplementary files. Reflectance spectra analyzed during the study are available from the corresponding author on reasonable request.

Results

X-linked opsin variation in *Eulemur*

Opsin genotyping

Results of our opsin analyses identified variation in the presence of opsin alleles across *Eulemur* species, but not across populations within species (Fig. 3). Seven species of *Eulemur* were monomorphic for the M opsin (no L allele): *E. albifrons*, *E. cinereiceps*, *E. collaris*, *E. coronatus*, *E. fulvus*, *E. rufifrons*, and *E. sanfordi*. All sequence traces had the following amino acid combination for sites 180, 277, and 285, respectively: alanine, tyrosine, alanine (AYA, λ_{max} ~543 nm). All high-resolution melt curves shared similar shape and temperature profiles as those samples that were sequenced (N = 44 for exon 3; N = 65 for exon 5), indicating no variation. In contrast, all individuals in all populations of red-bellied lemurs (*E. rubriventer*) were monomorphic for the L opsin (no M allele). Sequence traces (N = 4 exon 3; N = 8 exon 5) showed the following three-site combination: alanine, tyrosine, threonine (AYT, λ_{max} ~558 nm). All samples exhibited similar shape and temperature profiles, indicating no variation. Both M and L alleles (AYA and AYT), and thus polymorphic trichromacy, were only found in *E. flavifrons* and *E. macaco*. These were confirmed by sequence traces (N = 4 for exon 3; N = 6 for exon 5) and high-resolution melt curves. Consensus sequences for each species are available in ESM_2 and ESM_3 (for polymorphic species, the A-G nucleotide polymorphism is coded as R).

Ancestral color vision estimation and allele loss

The results of the stochastic character mapping analyses using two transition-rate patterns (symmetrical rates and different rates) are similar (Fig. S2). Accordingly, we report results for the analysis allowing transition rates to differ. Our results support either the M opsin or polymorphic trichromacy (M and L opsin) as the ancestral state for the genus *Eulemur* (posterior probability of M opsin only = 0.72, posterior probability of polymorphic trichromacy = 0.28, Fig. 3). Loss of polymorphic trichromacy likely occurred in one (*E. rubriventer*) or more *Eulemur* species.

Polymorphic trichromacy was the most likely ancestral state for the family Indriidae and the genus *Propithecus*, with apparent M opsin loss in the nocturnal genus *Avahi* (Fig. S2). Deeper nodes in the tree are estimated with less certainty, either as the M opsin or polymorphic. For example, the ancestral state for all Lemuridae has near-equal posterior probabilities of being either monomorphic with an M opsin (0.48) or

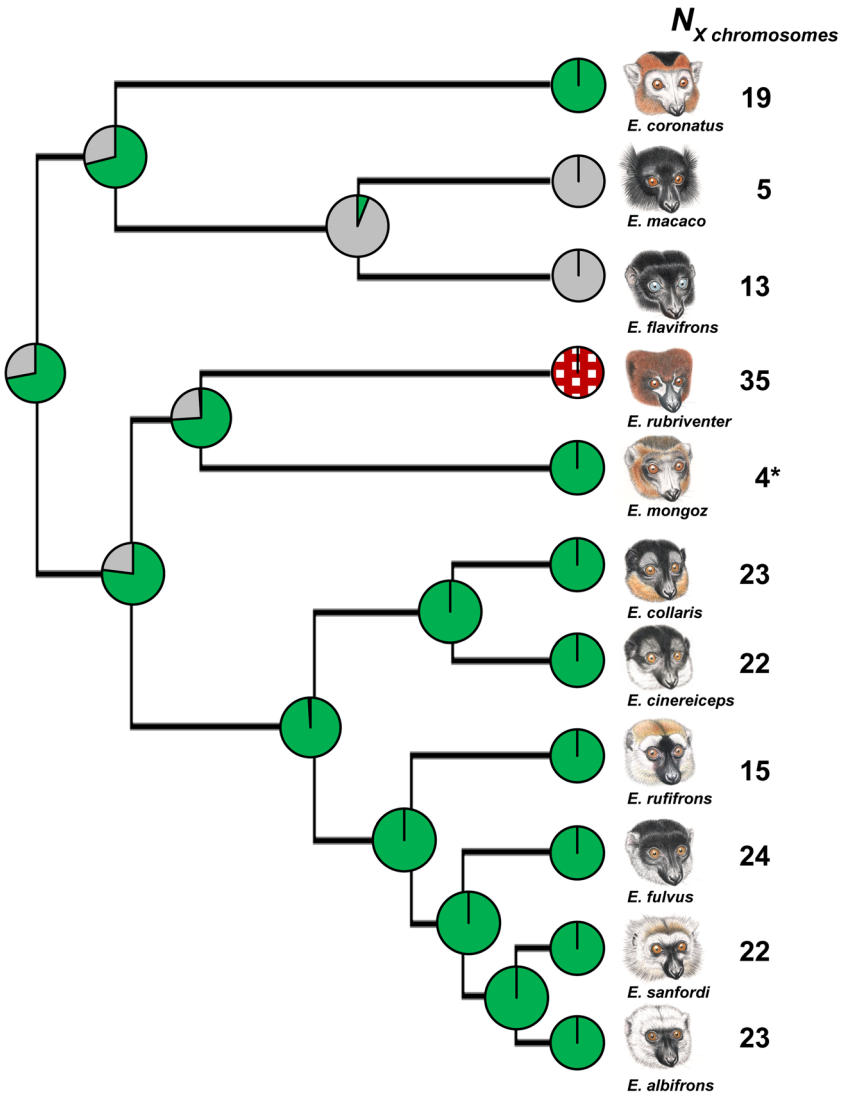

Fig. 3 Opsin gene variation and ancestral state estimations based on stochastic character mapping analyses for the genus *Eulemur*. Green circles at the tips represent the presence of the M opsin only (λ_{max} ~ 543 nm), the red patterned circle represents the presence of the L opsin only (*E. rubriventer*), (λ_{max} ~ 558 nm), and gray circles at the tips represent the presence of both M and L opsins (polymorphic trichromacy). Pie charts at each node represent the posterior probabilities for cone opsin ancestral states (colors defined as for the tips). Phylogeny from Herrera and Dávalos (2016). *Indicates data from published material (Tan and Li 1999). Illustrations copyright 2015 Stephen D. Nash/IUCN SSC Primate Specialist Group. Used with permission

polymorphic (0.47; posterior probability of L opsin only = 0.05). State changes most frequently occurred from the polymorphic state to the M opsin (10.6 transitions, on average across 10,000 simulations) or vice versa (8.93 transitions), and less frequently from the polymorphic state to the L opsin (8.45 transitions) or from the L opsin to the polymorphic state (4.27). Across the tree, there were on average 19 independent losses of an opsin allele. Lineages spent 51% of the time in the M opsin state, 31% of the time in the polymorphic state, and 18% of the time in the L opsin state.

Foraging ecology of *Eulemur rubriventer*

E. rubriventer food items

We recorded a total of 2924 foraging bouts on plant material during the study period. Table S2 lists all species and plant parts consumed, as well as the percentage of foraging bouts for each plant taxon. Overall, the nine groups of *E. rubriventer* fed on 115 plant taxa. Fruit foraging accounted for the majority of bouts (1947; 67%), followed by flowers/flower buds (480; 16%), and leaves (399; 14%). For the fruit foraging bouts for which ripeness of food items consumed could be determined (including $N = 58$ species), unripe fruit accounted for 56% of bouts.

Visual modeling analyses

We included reflectance data for 40 species (72 plant parts; see Fig. S3, for example, spectra) consumed by RNP red-bellied lemurs in our analyses (Table S2). The 40 species represent 75% of observed foraging bouts.

Chromaticity analyses Chromaticity plots for a trichromatic *Eulemur* under "day," "dusk," and "moonlit night" conditions are illustrated in Fig. 4 (see also Fig. S4). Red-green (L/(L+

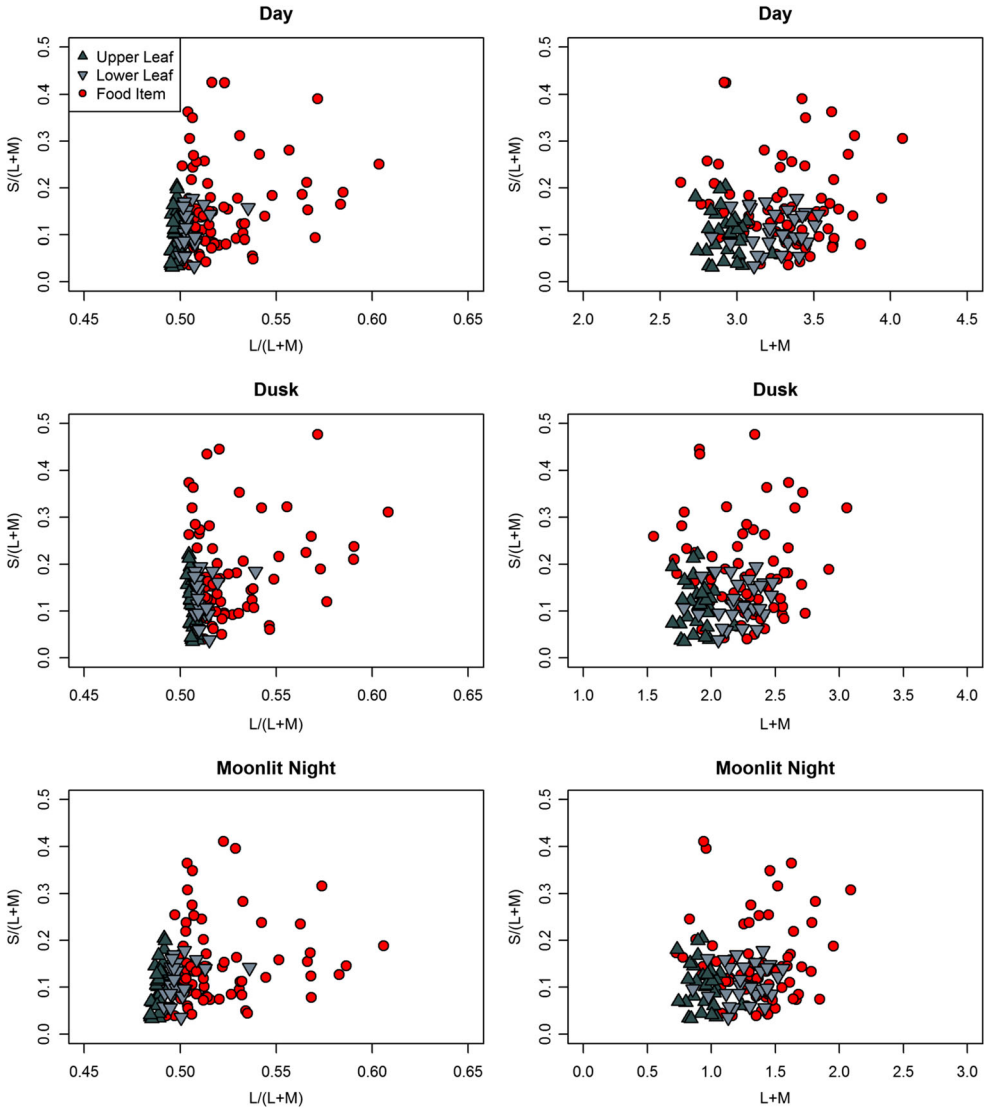

Fig. 4 Chromaticity and luminance plots under "day," "dusk," and "moonlit night" illuminations for 72 plant parts from 40 plant species consumed by *E. rubriventer* in RNP. Mean value is plotted for each plant part. Left, red-green (L/(L+M)) chromaticity vs. blue-yellow (S/(L + M)) chromaticity plots; right, luminance (L+M) vs. blue-yellow (S/(L+M)) chromaticity plots; S opsin $\lambda_{max} = 413$ nm, M opsin $\lambda_{max} = 543$ nm, L opsin $\lambda_{max} = 558$ nm

M)) vs. blue-yellow (S/(L+M)) chromaticity plots reveal that most food items have greater red-green chromaticity compared to mature leaves under all illumination conditions. Results of Mann-Whitney *U* tests reveal that red-green chromaticities of food items are significantly greater than mature leaves under all illuminations ("day," upper leaf $W = 2084$, $p < 0.001$; lower leaf, $W = 1741$, $p < 0.001$; "dusk," upper leaf, $W = 1974$, $p < 0.001$; lower leaf, $W = 1613$, $p < 0.001$; "moonlit night," upper leaf, $W = 2086$, $p < 0.001$; lower leaf, $W = 1783$, $p < 0.001$; Fig. 5; See Table S3 for descriptive statistics). Results hold under a Bonferroni corrected significance level ($\alpha < 0.008$).

Our results suggest that many food items with greater red-green chromaticities are ripe fruit (Fig. S4). To explore this further, we performed a post hoc Mann-Whitney *U* test to determine if ripe fruit ($N = 21$) has significantly greater red-green chromaticity than all other food items ($N = 51$). We found that the red-green chromaticity of ripe fruit is greater than that of other food items under "day" ($W = 232$, $p < 0.001$), "dusk" ($W = 257$, $p < 0.001$), and "moonlit night" ($W = 223$, $p < 0.001$) conditions.

Luminance (L+M) vs. blue-yellow chromaticity plots indicate that food items largely overlap with mature leaves in luminance as well as in blue-yellow chromaticity (Fig. 4, Fig. S4). A similar pattern of overlap in luminance and blue-yellow chromaticity for a trichromatic lemur is found in both dichromatic phenotypes (Fig. S5). Despite the large amount of overlap apparent from the chromaticity plots, for a trichromatic *Eulemur*, blue-yellow chromaticity is also significantly greater for food items compared to mature upper leaves ("day," $W = 1453$, $p < 0.01$; "dusk," $W = 1492$, $p < 0.001$; "moonlit night," $W = 1393$, $p < 0.01$). However, results do not hold for "moonlit night" conditions under Bonferroni correction ($\alpha < 0.008$). Compared to lower leaves, blue-yellow chromaticity is significantly greater under some light levels ("day," $W = 1367$, $p < 0.05$; "dusk," $W = 1405$, $p < 0.01$;

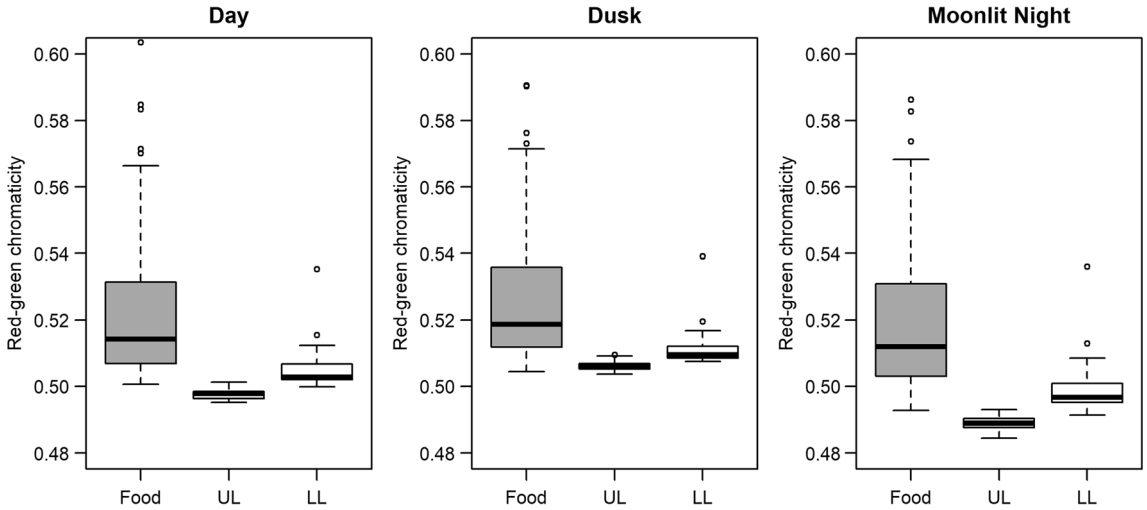

Fig. 5 Box-and-whiskers plots of red-green chromaticities (L/(L+M)) of food items, upper leaves (UL), and lower leaves (LL) under the three illumination conditions modeled in this study ("day," "dusk," and "moonlit night"); M opsin $\lambda_{max} = 543$ nm, L opsin $\lambda_{max} = 558$ nm. Horizontal lines within each box indicate the median of the distribution. Boxes envelop the interquartile range (50% of values) of the sample distribution, and whiskers encompass 1.5 times the interquartile range

"moonlit night," $W = 1301$, $p = 0.054$), but results only hold for "dusk" under Bonferroni correction ($\alpha < 0.008$). Luminance is significantly greater for food items compared to mature upper leaves ("day," $W = 1842$, $p < 0.001$; "dusk," $W = 1824$, $p < 0.001$; "moonlit night," $W = 1902$, $p < 0.001$), but not lower leaves ("day," $W = 1239$, $p = 0.144$; "dusk," $W = 1214$, $p = 0.203$; "moonlit night," $W = 1296$, $p = 0.059$; Bonferroni correction, $\alpha < 0.008$).

Contrast analyses Chromatic and luminance contrasts of food items differ between the two dichromatic phenotypes (Tables 3 and 4). Blue-yellow chromatic contrasts are greater for dichromats with the M opsin than chromatic contrasts based on the L opsin. This pattern holds under "day," "dusk," and "moonlit night" conditions ($N = 72$; upper leaf and lower leaf (all conditions), $p < 0.001$; see Table 3 for descriptive statistics), as well as under Bonferroni correction ($\alpha < 0.008$). Luminance contrasts, on the other hand, are significantly greater for dichromats with the L opsin compared to dichromats with the M opsin under all illuminants when viewed under upper leaf conditions ($N = 72$; upper leaf (all conditions), $p < 0.001$; Table 4), but not lower leaf conditions ("day," $V = 1168$, $p = 0.414$; "dusk," $V = 1336$, $p = 0.904$; "moonlit night," $V = 1092$; $p = 0.214$; Bonferroni correction, $\alpha < 0.008$).

JND analyses Results of the JND analyses indicate that a higher proportion of food items exhibit ≥ 1 chromatic JND value for trichromatic *Eulemur* compared to dichromatic *Eulemur* under "day" and "dusk" conditions. The proportions of food items that exhibit ≥ 1 chromatic JND value are similarly low (≤ 10%) for all color vision phenotypes under "moonlit night" conditions (Table 5). The proportions of food items with ≥ 1 chromatic JND value are similar for both dichromatic phenotypes, but dichromats with the L opsin exhibit the lowest proportions under all illumination conditions. Most food items (≥ 60%) have ≥ 1 luminance JND value for all color vision phenotypes, and proportions are similar across phenotypes under each illumination condition (Table 5).

Trichromats have significantly higher chromatic JND values compared to dichromats with the M opsin ("day," upper leaf, $V = 22$, $p < 0.001$; lower leaf, $V = 99$, $p < 0.001$; "dusk," upper leaf, $V = 8$, $p < 0.001$; lower leaf, $V = 0$; $p < 0.001$; "moonlit night," upper leaf, $V = 2$, $p < 0.001$; lower leaf, $V = 1$, $p < 0.001$) and dichromats with the L opsin ("day," upper leaf, $V = 0$, $p < 0.001$; lower leaf, $V = 4$, $p < 0.001$; "dusk," upper leaf, $V = 0$, $p < 0.001$; lower leaf, $V = 8$; $p < 0.001$; "moonlit night," upper leaf, $V = 0$, $p < 0.001$; lower leaf, $V = 0$, $p < 0.001$) (Fig. S6). Dichromats with the M opsin have significantly higher chromatic JND values compared to dichromats with the L opsin ("day," upper leaf, $V = 1963$, $p < 0.001$; lower leaf, $V = 2056$, $p < 0.001$; "dusk," upper leaf, $V = 1939$, $p < 0.001$; lower leaf, $V = 1860$; $p < 0.01$; "moonlit night," upper leaf, $V = 1992$, $p < 0.001$; lower leaf, $V = 2011$, $p < 0.001$) (Fig. S6). All results hold under Bonferroni correction ($\alpha < 0.0028$).

Luminance JND values (Fig. S7) are significantly greater under upper leaf conditions for dichromats with the L opsin compared to trichromats ("day," $V = 2284$, $p < 0.001$; "dusk," $V = 2269$, $p < 0.001$, "moonlit night," $V = 2521$, $p < 0.001$), and compared to dichromats with the M opsin ("day," $V = 396$, $p < 0.001$; "dusk," $V = 372$, $p < 0.001$; "moonlit night," $V = 124$, $p < 0.001$) (Fig. S7). Luminance JND values are significantly greater for trichromats compared to dichromats with the M opsin under upper leaf conditions ("day," $V = 444$, $p < 0.001$; "dusk," $V = 432$, $p < 0.001$; "moonlit night," $V =$

Table 3 Descriptive statistics of blue-yellow chromatic contrasts of food items ($N = 72$) against their leaf backgrounds (UL, upper leaves; LL, lower leaves) for the two *Eulemur* dichromatic phenotypes. Data are presented for the three illumination conditions modeled in this study. For reference, the blue-yellow chromatic contrast of a "reddish" ripe fruit (guava, Fig. S3) for a trichromatic phenotype under upper leaf and "day" conditions is 0.103 (red-green chromatic contrast, which is unavailable to dichromats is 0.082)

Background	Dichromat L opsin						Dichromat M opsin					
	Mean	SD	SE	25th %	Median	75th %	Mean	SD	SE	25th %	Median	75th %
Day												
UL	0.082	0.072	0.009	0.027	0.061	0.119	0.095	0.086	0.010	0.028	0.067	0.126
LL	0.071	0.069	0.008	0.023	0.048	0.094	0.084	0.081	0.010	0.029	0.059	0.103
Dusk												
UL	0.091	0.079	0.009	0.029	0.073	0.129	0.107	0.096	0.011	0.034	0.074	0.143
LL	0.079	0.074	0.009	0.026	0.057	0.108	0.095	0.090	0.011	0.033	0.068	0.129
Moonlit night												
UL	0.074	0.067	0.008	0.023	0.052	0.113	0.085	0.077	0.009	0.026	0.064	0.121
LL	0.065	0.063	0.007	0.022	0.045	0.083	0.075	0.073	0.009	0.024	0.050	0.095

221, $p < 0.001$). Significant results hold under Bonferroni correction ($\alpha < 0.0028$).

Luminance JND values are not significantly different for trichromats under lower leaf conditions compared to each dichromatic phenotype (M opsin, "day," $V = 1177$, $p = 0.444$; "dusk," $V = 1152$, $p = 0.365$; "moonlit night," $V = 1025$, $p = 0.106$; L opsin, "day," $V = 1530$, $p = 0.227$; "dusk," $V = 1561$, $p = 0.167$; "moonlit night," $V = 1637$, $p = 0.070$), nor are they significantly different between dichromats ("day," $V = 1140$, $p = 0.330$; "dusk," $V = 1124$; $p = 0.288$; "moonlit night," $V = 1003$, $p = 0.081$) (Fig. S7).

Discussion

Our results indicate that extant *Eulemur* species differ in color vision capacity, and only two species have a cone opsin polymorphism. Wild populations of *E. flavifrons* exhibit polymorphic trichromacy (as previously reported for a captive population; Veilleux and Bolnick 2009) as does its sister species, *E. macaco* (Fig. 3). Most other species of *Eulemur* appear to be monomorphic for the M opsin. *E. rubriventer* differs from all other congeners in being monomorphic for the L opsin. Variable distribution of opsin alleles occurs throughout the lemur tree and among closely related lemurids (i.e., *Varecia*—polymorphic, *Hapalemur*—L opsin, *Lemur*—M opsin; Tan and Li 1999), which might suggest a polymorphic ancestry in the genus *Eulemur*. However, our ancestral state estimations are more equivocal, with a higher probability of ancestral *Eulemur* being monomorphic for the M opsin based on a recent phylogeny. Together, these results suggest that polymorphic trichromacy was likely lost in at least one *Eulemur* species: *E. rubriventer*. Interestingly, our visual modeling analyses suggest that trichromatic color vision

Table 4 Descriptive statistics of luminance contrasts of food items ($N = 72$) against their leaf backgrounds (UL, upper leaves; LL, lower leaves) for the two *Eulemur* dichromatic phenotypes. Data are presented for the three illumination conditions modeled in this study. For reference, the luminance contrast of a "reddish" ripe fruit (guava, Fig. S3) for a trichromatic phenotype under upper leaf and "day" conditions is 0.061

Background	Dichromat L Opsin						Dichromat M Opsin					
	Mean	SD	SE	25th %	Median	75th %	Mean	SD	SE	25th %	Median	75th %
Day												
UL	0.425	0.262	0.031	0.227	0.429	0.584	0.399	0.252	0.030	0.219	0.384	0.563
LL	0.247	0.181	0.021	0.093	0.243	0.355	0.252	0.190	0.022	0.080	0.231	0.371
Dusk												
UL	0.415	0.260	0.031	0.227	0.414	0.579	0.397	0.253	0.030	0.202	0.389	0.551
LL	0.249	0.188	0.022	0.095	0.234	0.354	0.257	0.199	0.023	0.094	0.231	0.388
Moonlit night												
UL	0.457	0.263	0.031	0.236	0.478	0.609	0.414	0.258	0.030	0.217	0.399	0.579
LL	0.247	0.181	0.021	0.116	0.223	0.357	0.248	0.183	0.022	0.087	0.233	0.355

Table 5 Proportions of food items exhibiting ≥ 1 JND for each color vision genotype under each illumination condition and for upper and lower leaves (upper/lower). Results are presented for chromatic and luminance JNDs

Illumination	Trichromat	Dichromat M opsin	Dichromat L opsin
Chromatic JND			
Day	82/76	67/65	63/60
Dusk	47/49	38/36	33/35
Moonlit night	10/3	10/3	7/0
Luminance JND			
Day	94/92	94/90	93/90
Dusk	92/79	90/82	89/83
Moonlit night	78/64	76/63	81/65

would likely provide a foraging advantage to *E. rubriventer* during daylight and dusk conditions, which would seem to favor maintaining a cone opsin polymorphism. Although chromatic values for food items are comparatively higher for trichromatic *Eulemur* under all conditions, the proportions of food items that are chromatically conspicuous to *Eulemur* are similarly low for trichromatic and dichromatic individuals under moonlit night, indicating that trichromatic color vision is unlikely advantageous under nocturnal conditions. Moreover, we found that many food items should be conspicuous to dichromats in the absence of red-green color vision. Accordingly, given the cathemeral behavior of *E. rubriventer*, trichromatic color vision might not be under strong selection in this taxon, and fixation of the L opsin could therefore result from relaxed selection (genetic drift; Jacobs and Bradley 2016).

Our visual modeling analyses revealed intriguing differences between the two dichromatic phenotypes that could result in directional selection favoring the L opsin. Specifically, chromatic contrasts and chromatic JND values for *E. rubriventer* food items are significantly greater for dichromats with the M opsin compared to those with only the L opsin. On the other hand, luminance contrasts and luminance JND values are generally greater for dichromats with the L opsin compared to dichromats with the M opsin. Moreover, luminance JND values are generally greater for dichromats with the L opsin compared to trichromats. Therefore, we hypothesize that luminance is a more important foraging cue to *E. rubriventer*, favoring the adaptive L opsin.

Such an adaptation could be related to the particular diet of *E. rubriventer*, but other factors, such as activity pattern, might also play a role. For example, luminance vision may be particularly important under low light level conditions at night when chromatic conspicuity of food items is greatly reduced and the use of color vision may be precluded. *E. rubriventer* appears to have the lowest proportion of diurnal activity among cathemeral *Eulemur* for which data are available (Donati et al. 2016), which is consistent with this species relying more heavily on luminance vision. If luminance vision is highly relevant to *E. rubriventer*, then the L opsin, being superior for luminance vision compared to the M opsin, may have been fixed by natural selection.

In line with this hypothesis, *E. rubriventer* is restricted to rainforest environments, which under moonlit conditions appear to be richer in longer wavelengths compared to Madagascar's dry forest environments (Veilleux and Cummings 2012). The L opsin might therefore maximize photon absorption under these nocturnal rainforest conditions and thus allow for better luminance vision. Madagascar's dry forests, on the other hand, are comparatively richer in shorter and middle wavelengths under moonlight (Veilleux and Cummings 2012). Interestingly, unlike *E. rubriventer*, *E. mongoz*, some populations of *E. coronatus*, and at least some species of the "brown lemur complex" inhabit dry forests (Mittermeier et al. 2010). Although the M opsin appears to be the more likely ancestral *Eulemur* condition based on our analyses, indicating this trait may have been maintained in these lineages, it could also be adaptive for luminance vision under Madagascar's dry forest environments. Dichromatic color vision and fixation of different opsin alleles might therefore represent adaptations to nocturnal activity in different habitats. Resolving the ancestral color vision states and patterns of allele loss throughout the lemur lineage could help address this hypothesis.

If *E. rubriventer* color vision is related to a greater reliance on luminance vision, this presents another, non-mutually exclusive, hypothesis for potential loss of polymorphic trichromacy. Specifically, loss could result from selection against trichromacy. Previous research indicates that chromatic information corrupts luminance vision (Osorio et al. 1998). Although this is not a hypothesis we could formally test, as the effect is not accounted for in our visual modeling analyses, it likely explains why dichromatic primates exhibit greater foraging efficiency than trichromats on some camouflaged food items (Melin et al. 2007, 2010; Caine et al. 2010; Smith et al. 2012). For species that rely heavily on luminance vision, dichromacy may therefore have a net advantage leading to disruptive selection (i.e., selection against trichromacy). This raises the question why cathemeral *E. flavifrons* and *E. macaco* have polymorphic trichromacy, despite potential costs to luminance vision. As has been suggested by other researchers (Valenta et al. 2016), opsin variation could be related to differences in diet and/or activity patterns. For example, polymorphic trichromacy might be favored in species that have a high proportion of daytime activity (Valenta et al. 2016), or that spend large amounts of time foraging under high light levels (Yamashita et al. 2005). Although current comparative research does not necessarily support the former hypothesis (Donati et al. 2016), a population of polymorphic *Propithecus verreauxi* appears to feed at higher light levels compared to sympatric dichromatic *Lemur catta* (Yamashita

et al. 2005). Interestingly, the two polymorphic *Eulemur* species are known to inhabit a unique transitional environment between eastern rainforests and western dry forests (Sambirano) (Mittermeier et al. 2010), but whether this environment imposes a unique selective pressure is unknown.

As a final note, it is important to acknowledge that the psychophysical data available for lemur vision are limited. Chromatic and achromatic discrimination thresholds and data on cone ratios are currently lacking for lemurs (Olsson et al. 2018). These parameters can have a significant impact on the models used in our study (Olsson et al. 2018), which were designed for understanding chromatic processing separate from achromatic processing (Osorio and Vorobyev 2018). Accordingly, our results of the modeling analyses should be interpreted with caution and considered hypotheses to be tested as our understanding of lemur visual processing improves.

Moving forward, what is clearly needed, in addition to the psychophysical information above, is to examine the detailed foraging behaviors and visual environments of different *Eulemur* species/populations to test whether opsin variation seen across species is likely adaptive or neutral. Together with behavioral studies, determining whether (1) similar patterns in chromatic versus luminance conspicuity are observed for plant species consumed by dichromatic lemurs with the M opsin, and (2) trichromacy is advantageous to individual *E. flavifrons* and *E. macaco* would be highly instructive for assessing the evolutionary mechanisms underlying differences in color vision capacities.

Although we cannot definitively conclude that loss of opsin variation is adaptive for some lemurs, such as *E. rubriventer*, the results of this study underscore the importance of considering the many drivers of allelic variation and allele loss when assessing heterozygosity in conservation genetics. Loss of variation can potentially be adaptive and thus external efforts to increase diversity (e.g., via outcrossings; Johnson et al. 2010) could yield unintended consequences.

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Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

Ethical approval All behavioral protocols and animal handling procedures were approved by and adhered to institutional animal care requirements (Stony Brook IACUC# 2011-1895, Omaha's Henry Doorly Zoo and Aquarium IACUC# 97-001, 12-101, and Northern Illinois University IACUC #LA12-0011) and national laws. Data collection, sample collection, and export permits were obtained from Madagascar National Parks, formerly Association Nationale pour la Gestion des Aires Protégées (ANGAP), and the Ministère de l'Environnement, de l'Écologie et des Forêts. Samples were exported/imported under the Convention on International Trade in Endangered Species (CITES) Appendix I permits.

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