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Consequences of color vision variation on performance and fitness in capuchin monkeys

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CONSEQUENCES OF COLOR VISION VARIATION ON PERFORMANCE AND FITNESS IN CAPUCHIN MONKEYS

By

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Consequences of Color Vision Variation on the Performance and Fitness of Capuchin Monkeys

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The origin and maintenance of variation in natural populations are central to the study of evolution. When alternative alleles have obvious effects on phenotype and are common in a population, the maintenance of these alleles requires some form of balancing selection. Understanding how selection maintains multiple phenotypes in a population requires integration of genetic analyses of phenotypic differences with field studies on the performance consequences of these differences within an ecological context.

The color vision polymorphism characterizing most diurnal platyrrhine and strepsirrhine primates provides an excellent opportunity to investigate the maintenance of variation in natural populations. The polymorphism leads to multiple forms of color perception co-existing in a population. The mechanisms and behavioral consequences of this polymorphism are still hotly debated. The two main hypotheses for the maintenance are heterosis and some form of negative-frequency dependent selection. My dissertation evaluated the performance and fitness consequences of color vision variation within an ecological context in order to elucidate the mechanism maintaining variation at this locus.

In chapter one, I provide an introduction to the subject, as well as a synopsis of the results from my dissertation chapters. In chapters two and three, I examine the performance differences between dichromatic and trichromatic individuals in a highly controlled captive setting using ecologically-relevant detection tasks. My results demonstrate superior performance by trichromatic individuals, especially in low light conditions and amid complex visual tasks. In chapter four, I detail the success of a novel Taqman® probe used to determine opsin genotypes of capuchin monkeys (*Sapajus nigritus*); use of this probe enabled establishment of genotypes of wild capuchin monkeys sampled non-invasively. In chapter five, I examine the performance differences of dichromatic and trichromatic capuchin monkeys from a wild population when foraging for invertebrates. Trichromatic individuals demonstrated higher success rates than dichromatic individuals for total invertebrate captures and for cryptic invertebrates under all light conditions. There were no differences for non-cryptic prey. In chapter six, I examine fitness consequences of color vision variation in a wild population of capuchin monkeys. Trichromatic females weighed more and had higher birth rates than dichromatic females. Collectively, my research demonstrates clear and consistent advantages to trichromatic females from three distinct perspectives. My results support the heterosis hypothesis for the maintenance of the polymorphic visual system characteristic of New World primates.

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Chapter 1: Introduction

Primates are unique among mammals in possessing three distinct types of cone photoreceptors, an arrangement that supports trichromatic color vision (Jacobs 1996, Bowmaker 1998). Trichromacy is assumed most often to be adaptively linked to foraging tasks (Caine and Mundy 2000, Dominy and Lucas 2001, Osorio and Vorobyev 1996, Regan et al. 2001) although potential advantages for predator detection or social signaling have also been proposed as alternative selection pressures (Caine 2002, Kamilar et al. 2013, Sumner and Mollon 2003).

Despite these proposed advantages, trichromatic color vision is not universal among primates. Many platyrrhine (New World) primate species possess variable color vision systems featuring several distinct phenotypes present within a population (Jacobs 1996). A central question is what has maintained the presence of multiple color vision phenotypes for millions of years given the advantages to trichromacy? In response, some have suggested that different color vision phenotypes may be specifically adapted for different visual tasks or prove superior under certain viewing conditions (Melin et al. 2007, Osorio et al. 1998).

In this dissertation, I sought to understand the maintenance of this important phenotypic variation from an evolutionary and adaptive perspective. This requires understanding the links between genotype, phenotype, performance, and fitness. The link between genotype and phenotype has been completed by prior work (Mollon et al. 1984, Neitz et al. 1991, Shyue et al. 1998). Currently, the DNA from an individual can be genotyped at a few major sites responsible for the spectral shifts in cone photopigments and thus obtain an estimate of an individual's phenotype. In my research, the link between phenotype and performance was documented in captive animals in visual discrimination tests

using controlled stimulus parameters and in wild animals for routine foraging tasks in a natural setting. The phenotype to fitness link was also documented in a natural setting. I used long-term reproductive data as well as weight data from a wild population of monkeys in Iguazu, Argentina to assess the link between phenotype and fitness. My study adds to the existing literature on consequences of primate color vision variation by: 1) investigating phenotypic consequences of target detection using controlled and ecologically relevant stimuli; 2) investigating the phenotypic consequences of successful insect capture in a wild black capuchin (*Sapajus nigritus*) population in a subtropical rainforest; 3) investigating the fitness consequences of phenotypic variation in a wild black capuchin population; 4) the use of a novel sampling design to control for confounding variation when taking data from natural populations; 5) the use of modern statistical approaches; and 6) the development of novel real-time PCR probes to determine color vision phenotype from fecal samples collected noninvasively.

Color vision in primates

Color vision is defined as the ability to distinguish between objects based solely on differences in their wavelength composition. Color vision requires both multiple types of cone photoreceptors and neural mechanisms to compare the responses from the cone cells. In general, having more kinds of spectrally distinct cone cells increases the potential for neural comparision, in turn leading to an increase in the dimensionality of color vision. Different types of photoreceptors contain different photopigments whose spectral sensitivities are determined by a protein called opsin. For trichromatic color vision, the short-wavelength sensitive (S) pigment has peak sensitivity between 420 and 430nm and

middle-wavelength (M) and long-wavelength (L) sensitive pigments with peak sensitivities between 530 and 565 nm.

Two separate neural channels compare the outputs from the different cone types to support color vision. One compares the output from the S cones to the combined outputs of the L and M cones in an "on/off" fashion (Dacey 1994) while a second channel contrasts the respective outputs from M and L cones in a center-surround opponency fashion (Goodchild et al. 1996). In general, this second type of channel supports higher visual resolution than does the first. The amplitude of the chromatic signal depends on the separation of the spectral sensitivities of the cones and how many different cone types are present (Dacey 1996). The first channel, comparing the S cones to the combined outputs of the L and M cones, is present in most mammals. The second channel, comparing the outputs of the L and M cones, is unique to primates (Jacobs 1993).

All primates have a short-wavelength sensitive opsin encoded by an autosomal gene. A gene on the X chromosome encodes the M/L sensitive opsin. Ancestrally, primates exhibited a color vision similar to modern mammals with only two different cone types, an S sensitive opsin and one M/L sensitive opsin (dichromacy) (Jacobs 1993). The single M/L opsin gene on the X chromosome became polymorphic with alleles encoding different M and L opsin proteins (Jacobs and Neitz 1987). The allelic polymorphism of the X-linked opsin gene leads to the presence of several distinct color-vision phenotypes within a population. All males, having only a single X-chromosome, and those females with the same allele on each X-chromosome are dichromatic. Females with two different alleles on each X-chromosome are trichromatic (Jacobs and Deegan 2003). This type of color vision characterizes the majority of platyrrhine primates and a few diurnal strepsirhine species

(Jacobs 1998, Tan and Li 1999). The derived color-vision form in primates is often called "routine trichromacy". Routine trichromacy arose from two opsin genes being placed on the same X-chromosome, either because of a duplication event or unequal crossing over (Hunt et al. 1998). After the X-chromosome acquired two copies of the opsin gene they became fixed for two distinct opsin variants (Jacobs et al. 1996, Jacobs and Deegan 1999). With this arrangement, all males and females are routinely trichromatic with an S opsin gene on the autosome and both one M and one L opsin gene on the X-chromosome. All catarrhines exhibit this form of color vision in addition to one platyrrhine species, *Alouatta* (Jacobs et al. 1996, Jacobs and Deegan 1999).

Hypotheses for the maintenance of color vision variation in platyrrhines

Nearly all extant platyrrhine genera exhibit similar M/L opsin genes on the X-chromosome, and the amino acid differences at functionally critical sites between the alleles have been maintained with minimal variation for more than 20 million years (Boissinot et al. 1998, Hiwatashi et al. 2010, Hunt et al. 1998, Surridge and Mundy 2002). Evidence of strong balancing selection acting on the alleles at the sex-linked opsin gene suggests that the polymorphism is maintained by natural selection. Possible mechanisms for maintaining variation include spatial-temporal variance, heterosis, and negative frequency dependent selection. The spatial-temporal variance hypothesis seems very improbable in this scenario due to the fact that many platyrrhine species have the same color visual system regardless of differences in lifestyle and habitat requirements. Therefore, in my dissertation, I investigated the heterosis and negative-frequency dependent selection hypotheses for the maintenance of this long-term variation.

Heterosis occurs when an individual heterozygous at a locus has higher fitness than do individuals that are homozygous at that locus. For New World (NW) primate color vision, heterozygous individuals are those females with two different alleles on each X-chromosome. Under the heterosis hypothesis, the polymorphism at the X-linked opsin locus is maintained by an average long-term fitness advantage to the heterozygous (trichromatic) females relative to the homozygous (dichromatic) females. Although trichromatic individuals might not outperform dichromatic individuals in all tasks where color perception is important, it should be the case that trichromatic individuals have higher fitness than dichromatic individuals.

Negative-frequency dependent selection can maintain variation for a long period when rare phenotypes have a fitness advantage relative to the other phenotypes in the population. Under this hypothesis, selective pressures affect phenotypes differently. When applied to the color visual system of New World primates, this hypothesis postulates that the different color vision phenotypes in a population are not suboptimal but are maintained because they are adapted for different visual tasks. Therefore, dichromatic individuals are predicted to outperform trichromatic individuals at some tasks or under certain viewing conditions. Examples of proposed conditions and tasks where dichromats might outperform trichromats are when light levels are low or when searching for camouflaged prey (Caine et al. 2009, Melin et al. 2007, Morgan et al. 1992, Saito et al. 2005, Simunovic et al. 2001, Verhulst and Maes 1998). For example, when light is limited, the chromatic signal is not very useful and this could in some way interfere with luminance detection (Osorio et al. 1998, Perini et al. 2009). Similarly, when searching for a cryptic object against a complex background, trichromats might be distracted by color differences and thus not readily see

pattern changes or luminance differences. Dichromatic individuals, who cannot perceive the color difference as readily, might detect the pattern changes, edges, or contours (Morgan et al. 1992, Saito et al. 2005). Under this hypothesis, dichromatic individuals should outperform trichromatic individuals when foraging for cryptic invertebrate prey or under low light conditions. Although, at equilibrium, there should be no fitness difference between trichromatic and dichromatic females, either type should have the higher fitness when it is relatively rare in the population. Thus, dichromatic individuals should have higher fitness than trichromatic individuals when dichromatic individual are less frequent in the population than expected at equilibrium.

Earlier studies have examined aspects of these two hypotheses (see Table 1). As shown in Table 1, the results from these studies do not consistently support one hypothesis. Many of these studies did not show a difference between color-vision phenotypes, either due to small sample size or negligible effect size. Some of these studies conducted on performance differences between phenotypes lacked control conditions, easily-reproduced stimuli and/or provided tasks that were not ecologically relevant to the primate subjects. I designed my dissertation experiments to address the short-comings of previous work.

To try and elucidate the mechanism maintaining variation at the X-linked opsin locus, I evaluated the performance and fitness consequences of color vision variation from three perspectives within an ecological context. The first part of my research evaluated performance differences in a captive setting using controlled stimuli; the second part evaluated performance differences in the wild during routine foraging tasks while the third part evaluated fitness differences in the wild using long-term reproductive data and individual weight data.

In chapters two and three, I investigate the performance differences between dichromatic and trichromatic females in a highly-controlled captive setting, using ecologically-relevant detection tasks. Chapter 2 simulates differences in fruit finding under various environmental conditions with a colored target detection task against backgrounds varying in complexity under both bright and low light conditions. Chapter 3 simulates cryptic invertebrate foraging with cryptic insect targets presented against backgrounds varying in complexity under bright and low light conditions. In both target detection tasks, trichromatic individuals demonstrated superior performance relative to dichromatic individuals with the greatest differences seen under low light conditions and when the visual task was complex. These results lend support to the heterozygote advantage hypothesis for both colored and cryptic target detection in captivity.

Chapter 4 details the success of using a novel Taqman® probe to determine the opsin genotypes of wild capuchin monkeys (*Sapajus nigritus*) with real-time PCR from fecal samples collected non-invasively. The probe was successful in determining the single-nucleotide, base-pair changes at three important sites from fecal samples stored by various methods from as far back as 1995. The use of these probes for SNP analysis at the sex-linked opsin locus is likely applicable to other platyrrhine species.

In chapter five I investigate the performance differences between dichromatic and trichromatic capuchin monkeys (*Sapajus nigritus*) in routine foraging tasks from a wild population. I collected data on invertebrate capture success under various environmental conditions. The use of a novel sampling design to collect data provided greater power in determining differences between color vision variants by controlling for important sources of variation between samples such as habitat, time of day, light levels, group activity, etc. In

the wild, trichromatic females had significantly more invertebrate captures per hour than did dichromatic females. Furthermore, trichromatic females had higher success rates per hour on cryptic invertebrates, especially when foraging in low light conditions. When foraging on non-cryptic invertebrates, trichromatic and dichromatic females performed equally well. The data for this population are not consistent with the theoretical predictions and previous empirical findings of dichromatic foraging advantage under low-light conditions and on cryptic prey. Instead, they demonstrate a consistent advantage in invertebrate foraging for trichromatic individuals, thus supporting the heterozygote advantage hypothesis for the maintenance of polymorphic color vision.

Chapter six examines the fitness consequences of color vision variation using 20+ years of demographic data on a capuchin monkey (*Sapajus nigritus*) population, as well as weight data on females in the same population. Trichromatic females weighed significantly more than their dichromatic counterparts when controlling for age. Additionally, trichromatic females had higher birth rates than dichromatic females. There were no differences in survival between dichromatic and trichromatic females, but limitations on sample size would have made it essentially impossible to demonstrate significant selection on survival. Thus, two proxies of fitness lend support to the heterozygote advantage hypothesis for the maintenance of the color vision polymorphism in this population.

In summary, the results documented in my dissertation show a consistent advantage to trichromatic individuals under various conditions with different measures, thus supporting the heterosis hypothesis for the maintenance of color vision polymorphism in this population. If this mechanism is proved to be more general across other populations, it could help explain the long-term maintenance of the polymorphism and distinct alleles optimized for color

discrimination in 130+ species of NW primates. It also would help explain why duplication in howler monkeys and in the ancestor of Old World monkeys and apes quickly went to fixation conferring routine color vision on both males and females.

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Tables

Paper	HA	NFDS	Ambiguous	Species
Bunce et al. 2012			✓ (small sample size)	*Callicebus brunneus*
Caine and Mundy 2000	✓			*Callithrix geoffroyi*
Caine et al. 2003			✓ (small sample size)	*Callithrix geoffroyi*
Caine et al. 2009		✓		*Callithrix geoffroyi*
de Araujo et al. 2006	✓			*Saimiri sciureus*
Lucas et al. 2001	✓			Catarrhines spp.
Dominy et al. 2003			✓ (no effect shown)	*Saguinus imperator* and *fucsicollis*
Fedigan et al. 2014			✓ (no effect shown)	*Cebus capucinus*
Freitag and Pessoa 2012		(✓)		*Callithrix jacchus*
Hiramatsu et al. 2008			✓ (no effect shown)	*Ateles geoffroyi*
Jacobs 1990	✓	(✓)		*Saguinus fucsicollis* and *Saimiri sciureus*
Leonhardt et al. 2009			✓(small sample size)	Strepsirhine spp.
Morgan et al. 1992		✓		*Homo sapien*
Melin et al. 2007		(✓)		*Cebus capucinus*
Melin et al. 2008			✓(No effect shown)	*Cebus capucinus*
Melin et al. 2009	✓	(✓)		*Cebus capucinus*
Melin et al. 2010	(✓)	(✓)		*Cebus capucinus*
Osorio and Vorobyev 1996	✓			*Catarrhine spp.*
Osorio et al. 1998		(✓)		Catarrhine spp.
Osorio et al. 2004	✓			*Saguinus fuscicollis* and *mystax*
Perini et al. 2009	✓	(✓)		*Callithrix penicillata*
Regan et al. 2001	✓			*Alouatta seniculus, Ateles panicus,* and *Cebus apella*
Riba-Hernandez et al. 2005	✓			*Ateles geoffroyi*
Riba-Hernandez et al. 2004	✓			*Ateles geoffroyi*
Saito et al. 2005		✓		*Cebus apella, Macaca fasicularis, Pan troglodytes*
Smith et al. 2003a	✓			*Saguinus fuscicollis* and *mystax*
Smith et al. 2003b			✓ (no effect shown)	*Saguinus fuscicollis* and *mystax*
Smith et al. 2005	(✓)			*Saguinus fuscicollis* and *mystax*
Smith et al. 2012	✓	(✓)		*Saguinus fuscicollis, labiatus* and *mystax*
Stoner et al. 2005			✓ (no effect shown)	*Alouatta palliata* and *Alouatta geoffroyi*
Sumner and Mollon 2000a	✓			Catarrhine spp.
Sumner and Mollon 2000b	✓			Catarrhine spp.
Vogel et al. 2007			✓ (no effect shown)	*Cebus capucinus*
Yamashita et al. 2005			✓(equivocal results)	*Lemur, Propithecus, Ateles, Alouatta, Cecropithecus, Colobus, Piliocolobus*

Table 1. List of all published articles with data on the consequences of color vision variation. Papers either showed support for predictions following from the heterozygote advantage (HA) hypothesis, support for predictions from the negative-frequency dependent selection (NFDS) hypothesis, or were ambiguous in the results. Check marks under the HA column tended to be with colored food items or targets, whereas NFDS marks tended to be for cryptic food items or targets. A check mark in parentheses indicates a small effect shown or an effect under specialized conditions.

Chapter 2: Colored Target Detection in Trichromatic and Dichromatic Capuchin Monkeys

A.T. Green, C.H.Janson and M.Neitz

Introduction

It is a long-standing hypothesis that trichromacy evolved in primates to aid in foraging (Allen 1879, Mollon 1989, 1991, Polyak 1957). According to this idea, trichromatic individuals have better color discrimination and are therefore more efficient at detecting food items, such as fruits and young edible leaves, embedded in a mature canopy background that varies randomly in lightness and form (Dominy and Lucas 2001, Osorio and Vorobyev 1996, Regan et al. 1998, 2001, Sumner and Mollon 2000). Despite these potential advantages, not all primates are trichromats (Jacobs 1996). Nearly all species of New World monkeys have polymorphic color vision with several distinct color vision phenotypes co-occurring within a population (Jacobs 2007). This variable color vision system arises from allelic polymorphism at one X-linked opsin gene. The amino acid sequences of distinct opsins alter the spectral tuning of photopigments causing them to be preferentially tuned to different spectral locations. The short-wavelength sensitive [S] opsin gene is invariant and is located on an autosomal chromosome. A single, polymorphic middle/long-wavelength sensitive [M/L] opsin gene with three common allelic versions is located on the X-chromosome. The various possible combinations of the M/L pigments in an individual provide the basis for several discrete phenotypes and leads to all males and one third to one half of the females being dichromatic. The overall fraction of trichromats, about 25-33% of a given population, is very low compared to the over 99% rate of trichromacy found in Old World nonhuman primates

(Jacobs and Williams 2001, Onishi et al. 1999, 2002, Terao et al. 2005). Despite the relatively low frequency of trichromats in the New World primates, Hiwatashi et al. (2010) demonstrated strong balancing selection acting at the X-chromosome opsin locus for two genera of New World primates. The resulting high frequencies of the opsin alleles at this locus mean that the overall frequency of trichromatic females is often close to the maximum possible of 0.67 in a three-allele system.

The functional basis of this balancing selection remains unclear. The advantages to trichromatic individuals, especially those available when foraging for foods that signal palatability with a color change, remain the principal explanation in the literature (Caine and Mundy 2000, Melin et al. 2009, Osorio and Vorobyev 1996, Regan et al. 2001, Riba-Hernandez et al. 2004, 2005, Smith et al. 2003b, Sumner and Mollon 2000a, 2000b). However, many behavioral observations have produced results that are ambiguous or are in conflict with the predicted trichromatic advantage. For example, Caine and Mundy (2000) demonstrated an advantage to trichromats over dichromats at detecting orange targets at longer distances, but that difference disappeared at closer distances. Additionally, two separate studies conducted on tamarins found no consistent effect of color vision on either the nature of the leadership of the group to feeding sites or in their ability to locate feeding sites (Dominy et al. 2003, Smith et al. 2003a). Finally, in two different populations of capuchin monkeys, no differences were found between dichromats and trichromats in the time they spent foraging for different food types or in their foraging and energy intake rates (Melin et al. 2008, Vogel et al. 2007).

Alternative adaptive hypotheses for the presence of these color vision polymorphism postulate that natural selection facilitates the persistence of dichromatic individuals in the

population. These hypotheses predict that enhanced ability to distinguish chromatic differences in trichromats might interfere with performance in achromatic discrimination tasks (Morgan et al. 1992, Osorio et al. 1998, Perini et al. 2009). Thus, dichromacy might provide an advantage over trichromacy when chromatic differences do not provide any useful information, such as the perception of shapes, texture, depth perception, motion or under low-light conditions. Examples of tasks where dichromats might out-perform trichromats in the wild are when foraging on cryptic foods or detecting cryptic predators, or when foraging under low light levels.

Studies with human subjects demonstrated potential selective advantages to dichromatic individuals when detecting color-camouflaged objects and by possessing lower light perception thresholds (Morgan et al. 1992, Simunovic et al. 2001, Verhurlst and Maes 1998). Similar advantages were found for non-human primates in captivity and in the wild (Caine et al. 2003, 2009, Melin et al. 2007, Saito et al. 2005). Experiments using capuchin monkeys (*Sapajus apella*) and marmosets (*Callithrix geoffroyi*) to detect color-camouflaged objects have suggested a disadvantage to trichromats (Caine et al. 2003, Saito et al. 2005), and a field study found an advantage to dichromats in foraging for surface dwelling insects (Melin et al. 2007). In addition Caine et al. 2009 suggested a foraging advantage to dichromats in low-light intensity conditions. These observations suggest that the selective advantage conferred to trichromats by their enhanced ability to differentiate colors in the red-green spectrum may be mitigated by use of other visual cues and/or sensory modalities by dichromats to compensate for their inferiority in color sense. In fact, two studies by Hiramatsu et al. (2008, 2009) demonstrated that other modalities -- luminance cues and

olfactory inspection of fruit -- were important determinants to both trichromats and dichromats for detection and ingestion of fruits.

In this experiment we measured the performance of dichromatic and trichromatic capuchin monkeys (*Sapajus apella*) to detect circular targets for which the chromatic contrast of the target supplied the relevant cues. To do so, we evaluated the performance of captive dichromatic and trichromatic female capuchin monkeys using highly-controlled stimuli presented on a computer under various test conditions.

Methods

We carried out target detection experiments on three socially-housed groups of capuchin monkeys *(Sapajus apella*) in the United States (NIH, Alpha Genesis Inc., and Yale University). All behavioral experiments involved the use of a 17-inch touch sensitive screen accessible to the monkeys from their home cages. Small enclosures surrounding the touch sensitive screen ensured that only one monkey at a time could access the testing device and prevented any glare from ambient light sources on the screen. We trained female subjects to respond to stimulus targets using operant conditioning with positive reinforcement with a food reward. To mimic natural conditions typically encountered by foraging monkeys, we plotted natural insect and leaf reflectance measurements obtained from Nathaniel Dominy and other published sources (Regan et al. 2001) in a standard chromaticity diagram modified for the *Sapajus* eye. We calculated quantum catches for the four common cone sensitivities (S – 430 nm, M- 530nm, Ma – 550nm, L-562nm) in *Sapajus* for each stimulus spectrum (canopy background and target insects) using a color space model similar to Sumner and Mollon (2000) with absorbance curves for lenses and optical densities of macular pigment

from the literature (Tovee et al. 1992, Wyszecki and Stiles 1982). These quantum catch values were plotted on a chromaticity diagram representing the neural inputs of the luminance channel and both the older and more recent color contrast subsystems (Fig. 2 and 3). Natural food items fell into seven major color areas. Using the hue, saturation, and brightness settings, we matched target hue to the average hue for each color area, kept saturation constant at 67% and brightness in the range of 60 to 70% while still representing the chromatic properties of food items consumed by wild primates.

To test if visual phenotypes differ in target detection, we presented the monkeys with distinct colored "fruit" targets in random order against various backgrounds ranging in complexity from a plain non-textured background to a leaf-textured background. An example of the target detection tasks presented to the test subjects is shown in Figure 1. The embedded items were presented against three backgrounds (non-textured white, non-textured green, leaf-textured monochromatic green background) under two light conditions (bright and dim). The light conditions were manipulated by adjusting the brightness on the computer screen to its highest and lowest settings. Luminance changed approximately ten-fold between the dim and bright settings (15 cd/m^2 and 146 cd/m^2, respectively). Each experiment had eight targets of different colors covering approximately 5% of visual field. Two randomly selected targets were partially obscured by a leaf shape. The combination of background and light condition was constant for a given experimental trial.

Each experimental trial was set up using The Primate Vision Program created by Christopher A. Green at John Hopkins University. This is a Java Swing program that uses configurable visual displays to collect information from test subjects as they interact with the touch-screen display running the program. The displays presented to the test subjects are

configurable via a XML document that stipulates a background image along with smaller image buttons placed on top of the background (all configurable). When a test session is initiated, from a menu-choice or a command line argument, temporal-based information is written to a file. The test subject's identity, the experimental condition used for the test session, and each image button's click time are collected in this session log file. We collected data on the rate of detection and the number and color of detected and undetected targets under the various conditions.

We determined genotypes of the individuals by amplification and sequencing of the X-linked opsin gene at the three amino acid substitutions at positions 180, 277, and 285, which are important for spectral tuning (Neitz et al. 1991, Shyue et al. 1998). We extracted DNA from hair samples from each individual capuchin using a QIAamp DNA mini-kit (Qiagen, Crawley, UK). We identified trichromatic females by the presence of heterozygous sites in the DNA sequence at these important positions. We completed the genotyping of individuals after behavioral data collection to prevent any bias by the observer. We trained nine captive, adult female individuals to use the touch screen testing device. The ability of each monkey was built up in steps from touching the screen to touching multiple targets on the screen before receiving a food reward. The location and visual phenotype of all tested individuals are shown in Table 1. NIH and AGI colonies were outside under shaded conditions and all tests took place during the mid-day hours. The Yale colony was inside under typical office lighting.

We used a generalized linear mixed model fit by maximum likelihood with either a categorical or continuous dependent variable, using the LME4 package in R and the 'Fit Model' platform in JMP, respectively (R v. 3.02: The R Foundation for Statistical

Computing, 2013, and JMP v. 10.2, SAS Institute). Initial multifactorial models included all main effects and predicted interaction effects. If the initial model was significantly different from the null hypothesis (all main effects and interactions have zero effects, except for the grand mean of the dependent variable), backwards stepwise selection was performed to obtain a final model using the smallest number of significant ($P < 0.05$) predictors. The main fixed effects included brightness (two levels), background (four levels), color vision phenotype (two levels), and individual identity as a random effect. Within a phenotype, there was no difference in performance under bright and dim light when against the white or plain green backgrounds; therefore the data for bright and dim light levels within the white and green backgrounds have been combined for graphical simplicity in the diagrams below.

Results

There were no differences in performance between dichromats and trichromats for the conditions involving plain white backgrounds (Fig. 4, 5, and 6). Where the contrast between target and background was greatest, trichromats and dichromats performed equally well in detecting the targets. All individuals were able to find all eight targets on average and did so at an average rate of approximately 1 target per second. The number of targets found decreased for both phenotypes when the visual task was more complex, with dichromatic individuals affected more than trichromats (Figure 4). When detecting targets against the leaf-textured backgrounds for both the bright and dim light conditions, trichromatic individuals found more targets on average than the dichromats ($F_{3,29}=140.77$, $P<0.0001$). In the dim condition, trichromats had an average detection rate over twice as fast as the dichromats (Figure 5, Tri=0.539 targets/sec, Di=0.216 targets/sec; $F_{3,29}=29.62$, $P<0.0001$)

and found on average two more targets per trial than the dichromats. Light level explained very little of the variation in the results for the number of targets hit (removing light level decreases r^2 by only 0.021) or in the results for average detection rate between dichromatic and trichromatic individuals (0.0065 decrease in r^2)(Tables 2 and 3).

The search time needed to find the next target increased as the visual task became more complex, and this increase was more pronounced for dichromats than trichromats (Fig.6). Dichromats tended to have consistently longer search times than trichromats in the more difficult visual tasks (Table 4).

Individuals of different color-vision phenotypes were faster to choose targets of hues most suited to their discrimination abilities. Comparing the average wavelength of the first three targets hit and the last three targets hit in a trial, dichromats hit the shorter wavelength targets first in the more visually complex tasks (Fig. 7). This pattern was not seen with the dichromats in the white background experiments. Trichromatic individuals exhibited the opposite pattern, hitting longer wavelength targets first in the white and bright leaf-textured experiments.

Discussion

Under highly controlled conditions in our experiments, trichromatic individuals never showed poorer performance than did dichromatic individuals in finding fruit-like targets. Contrary to the hypotheses that postulate some kind of dichromatic advantage for the maintenance of variation in New World primate color vision, dichromatic individuals did not perform relatively better under visually complex conditions, but in fact did relatively worse.

Only under the least challenging conditions were dichromatic and trichromatic individuals equally effective at finding fruit-like targets.

In the literature on New World primate color vision, it has been very difficult to determine the costs and benefits of different color visual phenotypes in populations. This is mainly due to the multitude of confounding factors when measuring performance differences in both the wild and captive settings. If a phenotype is at a disadvantage relative to another phenotype for certain tasks, the disadvantaged phenotype might find ways to compensate for its disability by using other cues to complete the task (Hiramatsu 2008, 2009). We attempted to control for the other possible cues in our experiments in order to parse out the specific costs and benefits to different visual phenotypes by standardizing the luminance and saturation cues in the visual scenes and removing potential cues such as shape of an object from the target detection task. By controlling for these other cues, our results clearly show that trichromacy is linked to better performance at target detection when searching for colored targets amid a textured background. When the visual task is easy, such as searching for an object on a visually simple scene, dichromats are comparable to trichromats. This pattern is fully consistent with most theoretical analyses of the functions of trichromacy, that predict enhanced visual discrimination by trichromats of colored targets against complex backgrounds (Dominy and Lucas 2001, Osorio and Vorobyev 1996, Regan et al. 2001, Sumner and Mollon 2000a, 2000b).

It was surprising to see a markedly enhanced performance by the trichromats relative to dichromats when detecting targets in dimmer light conditions. We had expected to see the performance difference between dichromats and trichromats mitigated when performing under dim light conditions due to prior evidence in the literature (Perini et al. 2009, Verhulst

and Maes 1998). The performance by trichromats in the dimmer conditions seemed to suggest that color was very useful even when light was limited and cues other than color were well-controlled. Alternatively, the low-light conditions in our experiment may have been bright enough to still allow trichromats ready access to hue information.

The results from this experiment lend support to the trichromatic advantage hypothesis for the maintenance of this polymorphism in New World primate populations as it relates to detection of colored targets amid a textured background. The recent literature has pointed to other foraging tasks where dichromats outperform trichromats such as when foraging on cryptic insects especially in a light-limited environment. We will specifically address that task in a companion paper, in which we analyze performance differences between dichromats and trichromats under various simulated foraging conditions similar to those presented here.

Whether or not trichromatic individuals are uniformly superior to dichromatic ones for all natural visual tasks, the evidence presented here and the strong molecular evidence for balancing selection on M/L alleles is consistent with a net fitness benefit to trichromatic individuals. Such a net benefit explains the maintenance of the M/L polymorphism in all 130+ species of diurnal New World primates over at least 20 million years, except for the howler monkeys, which have routine trichromacy via a duplication of the M/L locus and subsequent fixation of different alleles at each locus (Hunt et al. 1998, Jacobs et al. 1996, Kainz et al. 1998). Although there may be situations in which dichromatic individuals excel in certain visual tasks relative to trichromatic ones (Caine et al. 2003, 2009, Melin et al. 2007, 2010, Morgan et al. 1992, Saito et al. 2005), the existence of such situations is not sufficient to explain the uniform maintenance of the M/L polymorphism. Such situation-

specific dichromat advantage needs to be coupled with negative frequency-dependent selection, with one outcome being that dichromats at least sometimes have demonstrably higher fitness than trichromats. Such an outcome has yet to be documented in New World primates (Fedigan et al. 2014).

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Tables

Social Group	Individual	Color Vision	Genotype
AGI	Polly	Tri	549/560
AGI	Sweetpea	Di	562/562
AGI	Zev	Tri	549/560
NIH	Destiny	Di	560/560
NIH	Irene	Tri	550/560
NIH	Snickers	Tri	550/560
YAL	Honey	Di	560/560
YAL	Jill	Tri	530/560
YAL	Mayday	Tri	530/560

Table 1. Details of individuals tested in this experiment

Source	N	DF	DFDen	F ratio	P>F
Visual Phenotype	1	1	6.02	4.5793	0.0760
Light Level	1	1	13438	86.6299	<.0001
phenotype*light level	1	1	13438	32.3893	<.0001
Background Complexity	2	2	13438	1256.885	<.0001
phenotype*background	2	2	13438	356.9983	<.0001
Light level*background	2	2	13438	76.2859	<.0001
phenotype*light level*background	2	2	13438	41.3650	<.0001

Table 2. Fixed Effects Tests for the number of targets hit per experiment model, R^2=0.31437
For details of the GLMM analysis and the main and random effects included, see Data Analysis.

Source	N	DF	DFDen	F ratio	P>F
Visual Phenotype	1	1	6.00	1.2364	0.3087
Light Level	1	1	13438	31.4629	<.0001
phenotype*light level	1	1	13438	118.5411	<.0001
Background Complexity	2	2	13438	5953.062	<.0001
phenotype*background	2	2	13438	101.2916	<.0001
Light level*background	2	2	13438	11.8058	<.0001
phenotype*light level*background	2	2	13438	46.5006	<.0001

Table 3. Fixed Effects Tests for the detection rate model. R^2= 0.687943 For details of the GLMM analysis and the main and random effects included, see Data Analysis.

Source	N	DF	DFDen	F ratio	P>F
Visual Phenotype	1	1	6.102345258	6.102345258	0.4341
Light Level	1	1	12725.162353	12725.162353	0.8397
phenotype*light level	1	1	12725.228301	12725.228301	0.0007
Background Complexity	2	2	12727.049533	12727.049533	<.0001
phenotype*background	2	2	12727.299345	12727.299345	0.0041
Light level*background	2	2	12725.38499	12725.38499	0.0607
Color of target	6	6	12725.161801	12725.161801	0.0073
Phenotype*color	6	6	12725.202921	12725.202921	0.9619
Light level* color	6	6	12725.083646	12725.083646	0.1836
Background*color	12	12	12725.103111	12725.103111	0.0981
Targets Remaining	1	1	12725.694362	12725.694362	<.0001
Phenotype*targets remaining	1	1	12725.742682	12725.742682	0.0003
Light Level*target remaining	1	1	12725.064732	12725.064732	0.0071
Phenotype*Light*Remain	1	1	12725.055789	12725.055789	0.0067
Background*targets remaining	2	2	12725.469955	12725.469955	<.0001
Phenotype*background*remain	2	2	12725.451835	12725.451835	0.0509
Color of Target*targets remaining	6	6	12726.173691	12726.173691	0.1850
Phenotype*color*remain	6	6	12726.165	12726.165	0.0028
Light level*color*remain	6	6	12725.386539	12725.386539	0.0140

Table 4. Fixed Effects Tests for the search time model. R^2= 0.272604 For details of the GLMM analysis and the main and random effects included, see Data Analysis.

Figures

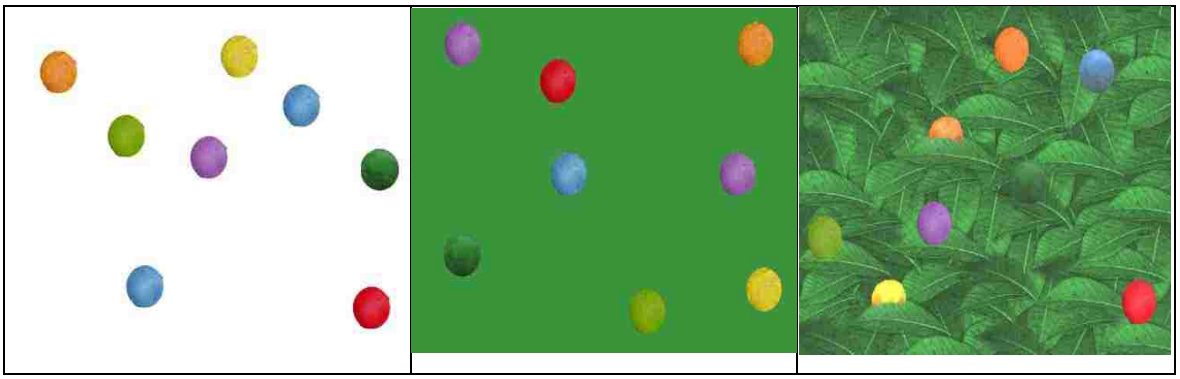

Figure 1. Example of the three experiments displayed under bright and dim light conditions. From left to right: non-textured white, non-textured green, leaf-textured monochromatic green backgrounds.

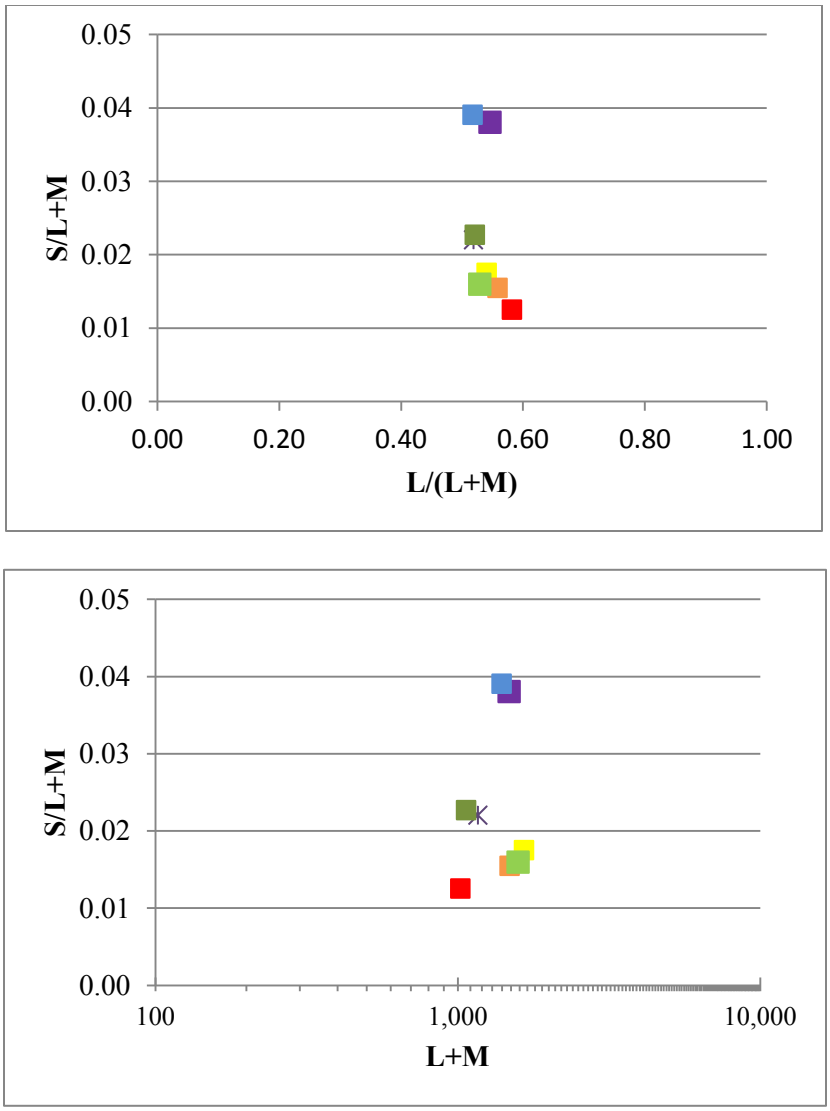

Figure 2. Standard chromaticity (top) and luminances (bottom) diagrams for targets and background (the peak sensitivities, λ_{max} , of the cone pigments were taken to be 430, 535, and 562nm, and the lens data from squirrel monkeys was used). The color of each marker represents the broad color category of the target as viewed by a human trichromat and the X marker represents the green background. The colors were chosen to be similar to measured reflectances of natural fruits, while constraining variation in luminance as much as possible.

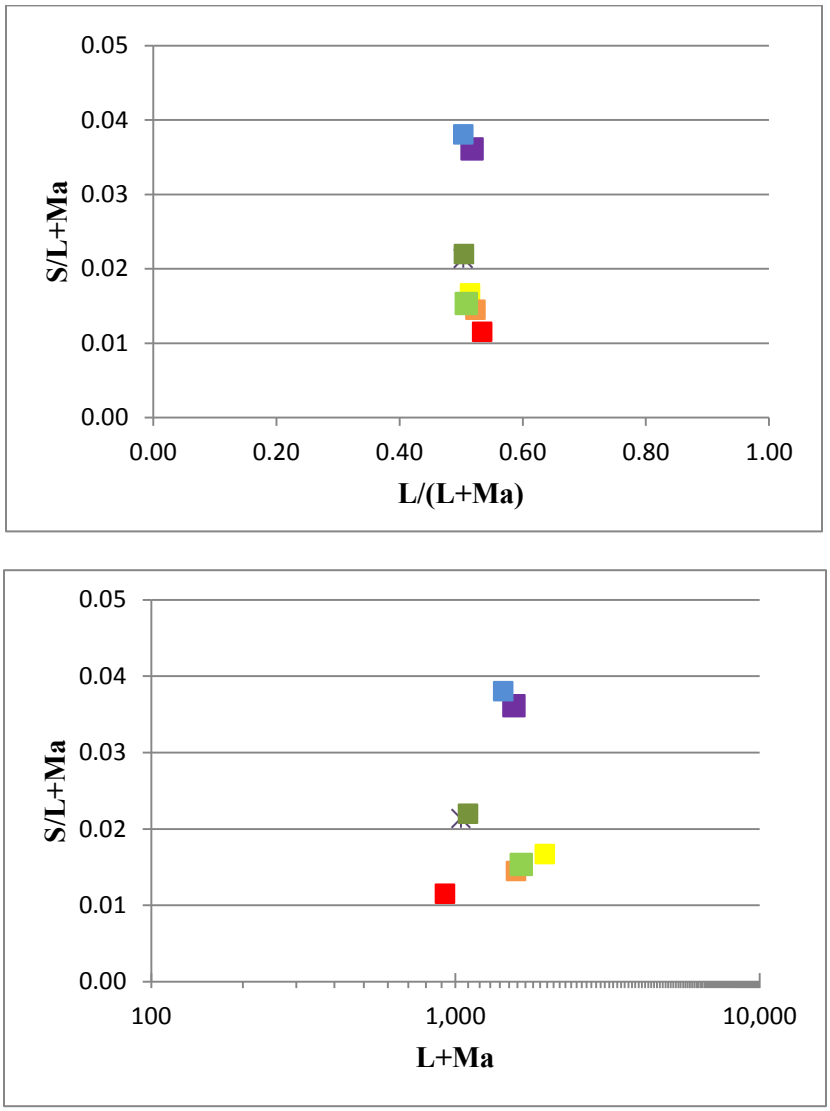

Figure 3. Standard chromaticity (top) and luminances (bottom) diagrams for targets and background (the peak sensitivities, λ_{max} , of the cone pigments were taken to be 430, 550, and 562nm, and the lens data from squirrel monkeys was used). The color of each marker represents the broad color category of the target as viewed by a human trichromat and the X marker represents the green background. The colors were chosen to be similar to measured reflectances of natural fruits, while constraining variation in luminance as much as possible.

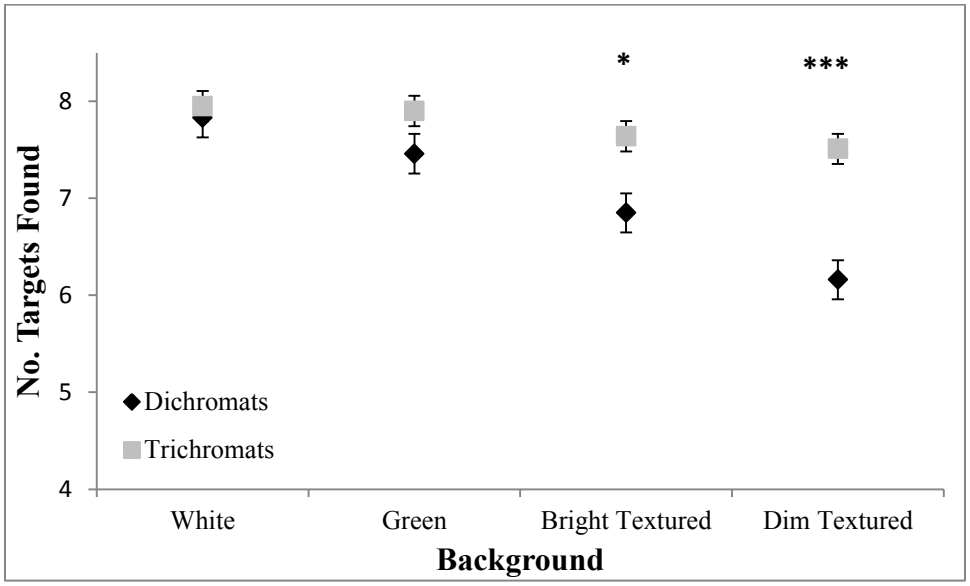

Figure 4. The average number of targets found per experiment for dichromats (n=3) and trichromats (n=6). Experimental conditions increase in complexity from left to right. The error bars represent +/- SE.

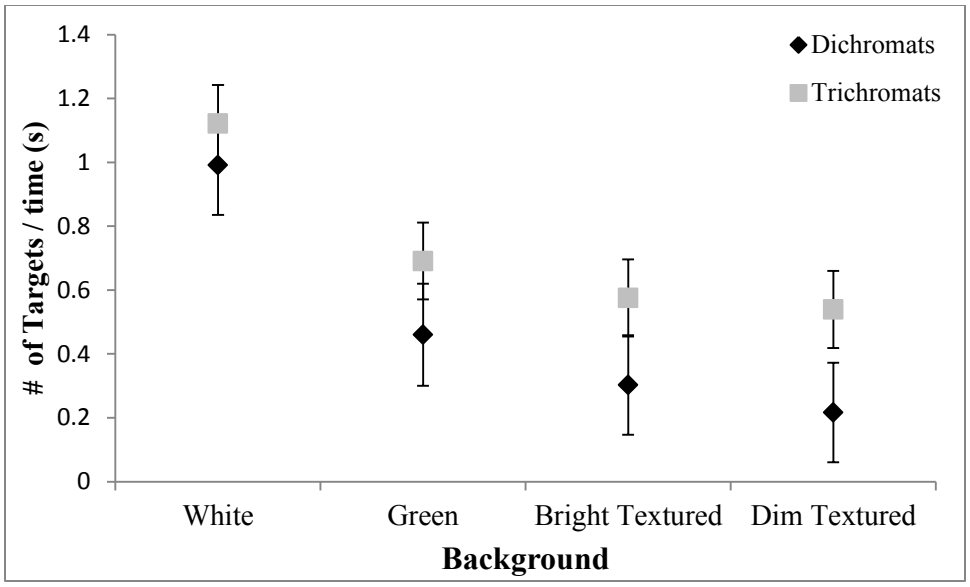

Figure 5. The average detection rate for each experiment. Error bars represent +/- SE.

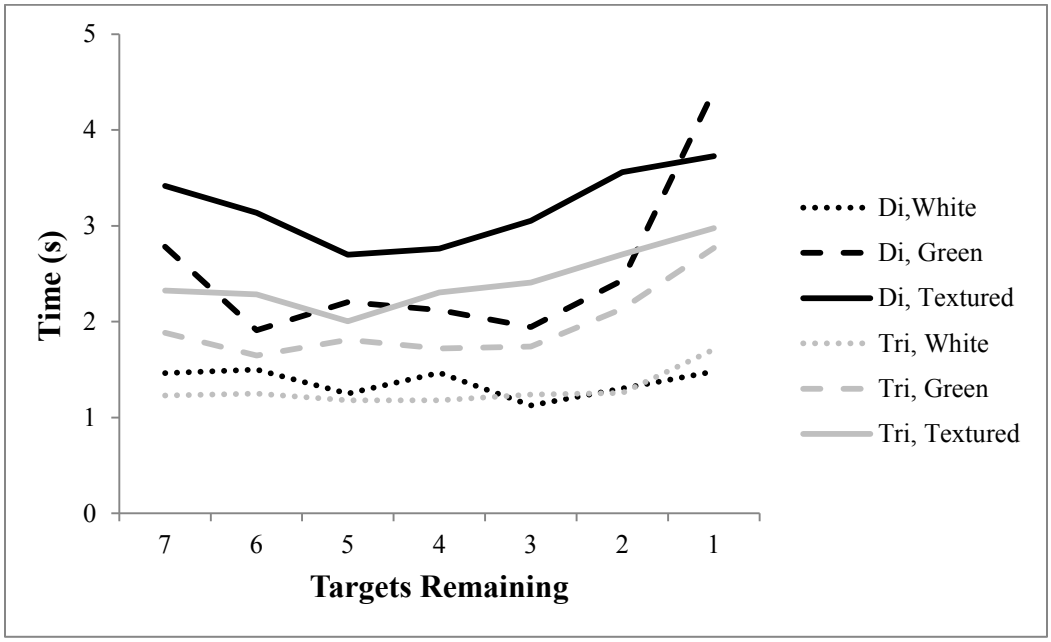

Figure 6. Search time to find the next target as a function of the number of targets remaining.

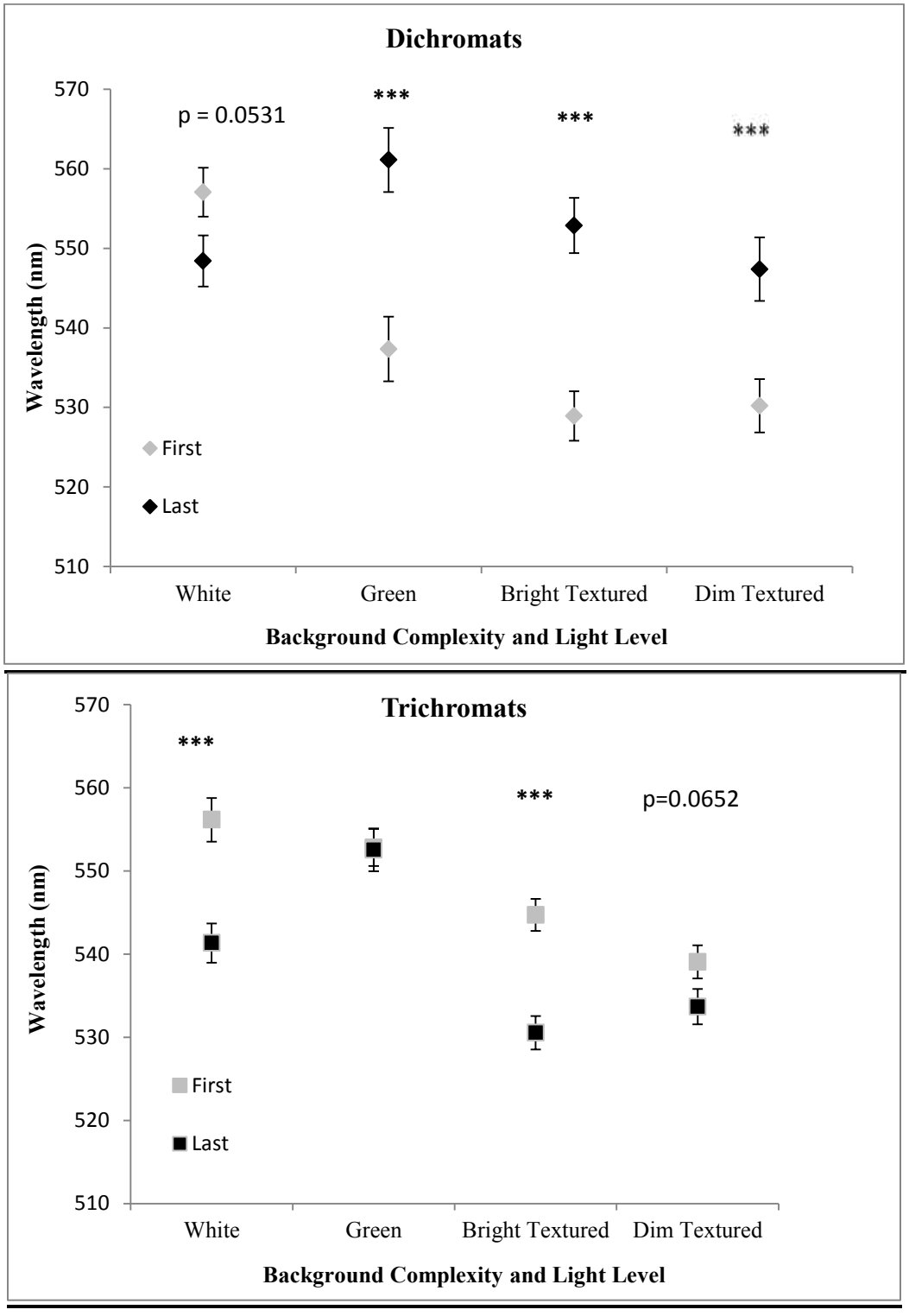

Figure 7. Average wavelength of the first and last three targets hit by dichromatic and trichromatic individuals

Chapter 3: Differences in camouflaged target detection between trichromatic and dichromatic monkeys for controlled, ecologically-relevant stimuli.

A.T. Green, C.H.Janson and M.Neitz

Introduction

It is a long-standing hypothesis that trichromacy evolved in primates to aid in foraging (Allen 1879, Mollon 1989, 1991, Polyak 1957). According to this idea, trichromatic individuals have better color discrimination and are therefore more efficient at detecting food items, such as fruits and young edible leaves, embedded in a mature canopy background that varies randomly in lightness and form (Dominy and Lucas 2001, Osorio and Vorobyev 1996, Regan et al. 1998, 2001, Sumner and Mollon 2000a, 2000b). Despite these potential advantages, not all primates are trichromats (Jacobs 1996). Nearly all species of New World monkeys have polymorphic color vision with several distinct color vision phenotypes co-occurring within a population (Jacobs 2007). This variable color vision system arises from allelic polymorphism at one X-linked opsin gene. The amino acid sequences of distinct opsins alter the spectral tuning of photopigments causing them to be preferentially tuned to different spectral locations. The short-wavelength sensitive [S] opsin gene is invariant and is located on an autosomal chromosome. A single, polymorphic middle/long-wavelength sensitive [M/L] opsin gene with three common allelic versions is located on the X-chromosome. The various possible combinations of the M/L pigments in an individual provide the basis for several discrete phenotypes and leads to all males and one third to one half of the females being dichromatic. The overall fraction of trichromats, about 25-33% of a given population, is very low compared to the over 99% rate of trichromacy found in Old

World nonhuman primates (Jacobs and Williams 2001, Onishi et al. 1999, 2002, Terao et al. 2005). Despite the relatively low frequency of trichromats in the New World primates, Hiwatashi et al. (2010) demonstrated strong balancing selection acting at the X-chromosome opsin locus for two general of New World primates. The resulting high frequencies of the opsin alleles at this locus mean that the overall frequency of trichromatic females is often close to the maximum possible of 0.67 in a three-allele system.

The functional causes of this balancing selection remain unclear. The trichromatic advantage hypothesis remains the principal explanation (Caine and Mundy 2000, Melin et al. 2009, Mollon et al. 1984, Osorio and Vorobeyv 1996, Regan et al. 2001, Riba-Hernandez et al. 2004, 2005, Smith et al. 2003b, Sumner and Mollon 2000a, 2000b). Under this hypothesis, the stable polymorphism is maintained by the consistent fitness advantage to trichromatic individuals in tasks such as foraging or predator detection. The trichromatic advantage hypothesis implies that the majority of the individuals in a given population, the ones who are dichromatic, have suboptimal fitness.

Alternative adaptive hypotheses to the trichromatic advantage hypothesis postulate that natural selection has facilitated the persistence of dichromatic individuals in the population. Under these hypotheses, it is predicted that there are visual tasks where having dichromacy would be an advantage over having trichromacy (Mollon et al. 1984). The theoretical basis for these hypotheses is that the enhanced ability to distinguish chromatic differences interferes with other visual abilities (Morgan et al. 1992, Osorio et al. 1998, Perini et al. 2009). Therefore, signals where chromatic differences do not provide any useful information, such as the perception of shapes, texture, depth perception, motion or under low-light conditions, are weakened by the two different spectral inputs of the L and M

photoreceptors in trichromats. Examples of tasks where dichromats might out-perform trichromats in the wild are when foraging on cryptic foods or detecting cryptic predators, or when foraging under low-light levels.

Studies with human subjects demonstrated potential selective advantages to dichromatic individuals when detecting camouflaged objects and possessing lower light perception thresholds (Morgan et al. 1992, Simunovic et al. 2001, Verhurlst and Maes 1998). Similar advantages were found for non-human primates in captivity and in the wild (Caine et al. 2003, 2009, Melin et al. 2007, Saito et al. 2005). These potential advantages to dichromats prompt the question of why trichromacy was rapidly selected for in Old World primates and why variant color vision phenotypes are so rare in non-human catarrhines (Jacobs and Williams 2001, Onishi et al. 1999, 2002, Terao et al. 2005).

In this experiment we measured the performance of dichromatic and trichromatic monkeys to examine whether an advantage exists for different visual phenotypes when searching for color-camouflaged targets such as surface-dwelling invertebrates. We evaluated the relative performance of captive dichromatic and trichromatic capuchin monkeys utilizing highly-controlled, ecologically-relevant stimuli under different viewing conditions to determine under which conditions certain visual phenotypes have an advantage. This study improves on previous approaches to this problem by increasing sample size, more consistent presentation of targets, and a closer approximation of natural foraging tasks.

Methods

We carried out target detection experiments on three socially-housed groups of *Sapajus* in the United States (NIH, Alpha Genesis Inc., and Yale University). All behavioral

experiments involved the use of a 17-inch touch sensitive screen accessible to the monkeys from their home cages. Small enclosures surrounding the touch sensitive screen ensured that only one monkey at a time could access the testing device and prevented any glare from ambient light sources on the screen. We trained female subjects to respond to perceived stimuli presented using operant conditioning with positive reinforcement. To mimic natural conditions typically encountered by foraging monkeys, we plotted natural insect and leaf reflectance measurements obtained from Nathaniel Dominy and other published sources (Regan et al. 2001) in a standard chromaticity diagram modified for the *Sapajus* eye. We calculated quantum catches for the four common cone sensitivities (S- 430 nm, M- 530nm, Ma -550nm, L-562nm) in *Sapajus* for each stimulus spectrum (canopy background and target insects) using a color space model similar to Sumner and Mollon (2000), with absorbance curves for lenses and optical densities of macular pigment from the literature (Tovee et al. 1992, Wyszecki and Stiles 1982). These quantum catch values were plotted on a chromaticity diagram representing the neural inputs of the luminance channel and both the older and more recent color contrast subsystems. The data are plotted on two diagrams: one representing the MacLeod-Boynton chromaticity diagram of L/(L+M) versus S/(L+M)(MacLeod and Boynton, 1979), and a plot representing the dichromatic color space of S/(L+M) versus luminance (L+M). Using the hue, saturation, and brightness settings, we matched hue to the average hue of natural stimuli, kept saturation constant at 67% and brightness in the range of 60 to 70% while still representing the chromatic properties of food items consumed by wild primates.

To test if there are differences between visual phenotypes in target detection, we presented the monkeys with various color-camouflaged “insect” targets presented against

various backgrounds. An example of the target detection tasks presented to the test subjects is shown in Figure 1. These were presented in random order and ranged in complexity from a plain non-textured background to a leaf-textured background. Cryptic targets were defined as targets that were indistinguishable from the chromatic properties of the background. For this reason, no chromaticity diagram is shown here, as the points for background and target lay on top of each other for each phenotype. The embedded items were presented against four backgrounds (non-textured "plain" white, non-textured "plain" green, leaf-textured monochromatic green, and leaf-textured, brown/green dappled "real" background) under two light conditions (bright and dim). The light conditions were manipulated by adjusting the brightness on the computer screen to its highest and lowest settings. The luminance from the computer screen changed approximately 10-fold between the dim and bright light settings (15 cd/m^2 and 146 cd/m^2). Each experiment had six targets of two sizes, with three being approximately 5% of visual field and three being 10% of visual field. Each experimental trial was set up using The Primate Vision Program created by Christopher A. Green at John Hopkins University. This is a Java Swing program that uses configurable visual displays to collect information from test subjects as they interact with the touch-screen display running the program. The displays presented to the test subjects are configurable via a XML document that stipulates a background image along with smaller image buttons placed on top of the background (all configurable). When a test session is initiated, from a menu-choice or a command line argument, temporal-based information is written to a file. The test subject's identity, the experimental condition used for the test session, and each image button's click time are collected in this session log file. Data were collected on the rate of detection and the number and color of detected and undetected targets under the various conditions.

Genotypes were determined by amplification and sequencing of the X-linked opsin gene at the three amino acid substitutions at positions 180, 277, and 285, which are important for spectral tuning (Neitz et al. 1991, Shyue et al. 1998). DNA was extracted from hair samples from each individual capuchin using a QIAamp DNA mini-kit (Qiagen, Crawley, UK). Trichromatic females were identified by the presence of heterozygous sites in the DNA sequence at these important positions. Genotyping of individuals was completed after behavioral data collection to prevent any bias by the observer.

Nine captive, adult female individuals were trained to use the touch screen testing device. The ability of each monkey was built up in steps from touching the screen to touching multiple targets on the screen before receiving a food reward. The test location and visual phenotype of all subjects are shown in Table 1. NIH and AGI colonies were tested outside under shaded conditions and all tests took place during the mid-day hours. The Yale colony was tested inside under typical office lighting.

For statistical inference, we used generalized linear mixed model fit by maximum likelihood with either a categorical or continuous dependent variable, using the LME4 package in R (v. 3.02: The R Foundation for Statistical Computing, 2013) and the 'Fit Model' platform in JMP (v. 10.2, SAS Institute), respectively. Initial multifactorial models included all main effects and predicted interaction effects. If the initial model was significantly different from the null hypothesis, backwards stepwise selection was performed to obtain a final model using to the smallest number of significant ($P < 0.05$) predictors. The main fixed effects included brightness (two levels), background (four levels), color vision phenotype (two levels), and target size (two levels), with individual identity as a random effect.

Results

There was no difference in performance between dichromats and trichromats in the plain background experiments for all measures analyzed (Fig. 2, 3, and 4). When the detection task was easy, as in the "plain" background experiments, trichromats and dichromats performed equally well in detecting the targets. They were able to find all six targets on average and do so at a rate of slightly more than 1 target per second. The difference in performance between dichromatic and trichromatic target detection became more evident when the visual task became more complex with the monochromatic textured background ("textured") and the more realistic canopy background with both texture and color differences ("real"). As seen in figure 2, the number of targets hit dropped for both phenotypes when the visual task was more complex to less than one target every five seconds. In both the textured and canopy realistic backgrounds, trichromatic individuals hit more targets on average than the dichromats (textured $F_{1,6}$=6.89, P=0.0396, real $F_{1,6}$=13.73, P=0.0099). Trichromats had an average detection rate over twice as fast as the dichromats (Figure 3, Tri=0.333 targets/sec, Di=0.153 targets/sec; $F_{3,29}$=3.39, P=0.0313) and found on average two more targets than the dichromats in the "real" conditions. The light level explained very little of the variation in the models for the number of targets hit (removing light level reduced r^2 by 0.05) or for the average detection rate between dichromatic and trichromatic individuals (when removed, light level reduced r^2 by 0.01; see Tables 2 and 3 for fixed effects tests).

The search time to find the next target increased as the visual task became more complex (Fig. 4). When the visual task was easy there was no difference between dichromats and trichromats in the time needed to find the next target (Table 4), but when the

visual tasks became more complex the time to the next target increased more for dichromats than for trichromats (textured $F_{1,9}$= 5.99, P=0.0374, real $F_{1,9}$=10.24, P=0.0107). The time to find the next target was also influenced by the size of the target and the background on which it was presented (Fig. 5). Search time increased when the target was smaller, with dichromats increasing more than trichromats ($F_{1,7}$= 4.84, P=0.0626) and also increased when the target detection task was presented under "real" conditions, with dichromats needing more time than trichromats to find the next target under those conditions ($F_{3,43}$=4.92, P=0.005). The difference between dichromats and trichromats in the time to find the next target was also larger when the light level was lower (Fig. 5).

Discussion

In previous studies, dichromatic individuals sometimes outperformed trichromatic individuals in certain conditions (Caine et al. 2003, 2009, Freitag and Pessoa 2012, Melin et al. 2007, 2010, Perini et al. 2009, Saito et al 2005). Two of these conditions are searching for camouflaged insects and foraging in low light conditions. Our evidence did not support those results with the detection tasks we tested. In our experiments, dichromatic and trichromatic individuals performed equally well under bright and dim conditions when the visual scene was simple, as in the plain, non-textured backgrounds. When the detection task was changed to present targets amid a monochromatic textured background or a more realistic color-variable textured background, this proved to be a difficult task for monkeys of all visual phenotypes and was even more difficult when light was limited. Under these conditions, the trichromatic individuals outperformed the dichromatic individuals: search times between targets were significantly lower for trichromatic individuals relative to the

dichromatic ones. These results are congruent with predictions from visual discrimination models that suggest that trichromats should be generally better than dichromats even in low light conditions (Osorio et al. 2004, Sumner and Mollon 2000, Regan et al. 2001).

A few studies done in a laboratory setting have shown that dichromats perform as well or better than trichromats in detecting cryptic targets (Caine et al. 2003, 2009, Morgan et al. 1992, Saito et al. 2005). Possible explanations for this difference from our results are 1) we controlled the visual scene to a great degree, decreasing non-chromatic and other possible cues that might be used by dichromats in nature, and 2) we used ecologically-relevant targets in the detection tasks. Because of the lack of luminance cues for distinguishing targets from the heterogeneous background in our experiments, it is likely that the targets appeared camouflaged to both the trichromatic and dichromatic subjects. In contrast, in the design of some previous detection experiments, the task was camouflaged to a trichromatic eye, but still contained useful cues available to the dichromatic eye, similar to standard Ishihara tests. Finally, only females were tested in this experiment, thus eliminating any confounding effect due to performance differences between males and females.

Our results lend support to the heterozygote advantage hypothesis predicting that trichromatic females, with different opsin alleles at each X-chromosome locus, have a fitness advantage due to superior visual discrimination abilities. Our results do not support the idea that dichromatic individuals outperform trichromatic individuals under certain conditions. Rather, trichromatic individuals exhibited superior performance especially in low light conditions and amid complex backgrounds. This superior performance by the trichromats under dim conditions in this experiment is consistent with functional trichromatic vision in

low light conditions based on other modeling, phylogenetic analysis, and field studies (Melin et al. 2013, Osorio et al. 2004, Smith et. al. 2012, Tan and Li 1999).

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Tables

Social Group	Individual	Color Vision	Genotype
AGI	Polly	Tri	549/560
AGI	Sweetpea	Di	562/562
AGI	Zev	Tri	549/560
NIH	Destiny	Di	560/560
NIH	Irene	Tri	550/560
NIH	Snickers	Tri	550/560
YAL	Honey	Di	560/560
YAL	Jill	Tri	530/560
YAL	Mayday	Tri	530/560

Table 1. Details of individuals tested in this experiment

Source	N	DF	DFDen	F ratio	P>F
Visual Phenotype	1	1	6.00	4.7117	0.0730
Light Level	1	1	9425	45.9825	<.0001
phenotype*light level	1	1	9425	23.4486	<.0001
Background Complexity	2	2	9425	8224.175	<.0001
phenotype*background	2	2	9425	508.5881	<.0001
Light level*background	2	2	9425	15.8531	<.0001
phenotype*light level*background	2	2	9425	20.7904	<.0001

Table 2. Fixed Effects Tests for the number of targets hit per experiment model. R^2=0.70631

Source	N	DF	DFDen	F ratio	P>F
Visual Phenotype	1	1	6.00	0.4711	0.5181
Light Level	1	1	9425	163.6880	<.0001
phenotype*light level	1	1	9425	0.4098	0.5221
Background Complexity	2	2	9425	4694.9064	<.0001
phenotype*background	2	2	9425	22.6506	<.0001
Light level*background	2	2	9425	98.5365	<.0001
phenotype*light level*background	2	2	9425	12.1011	<.0001

Table 3. Fixed Effects Tests for the detection rate model. R^2= 0.585169

Source	N	DF	DFDen	F ratio	P>F
Phenotype	1	1	6.00	4.5035	0.0742
Light Level	1	1	6110	0.1556	0.6933
Phenotype*light levels	1	1	6110	0.7491	0.3868
Background	2	2	6110	433.9934	<.0001
Phenotype *background	2	2	6110	25.4593	<.0001
Light levels *background	2	2	6110	8.9724	0.0001
Phenotype*light level*background	2	2	6110	0.1580	0.8539
Size	1	1	6110	0.3907	0.5319
Phenotype *size	1	1	6110	0.4593	0.4980
Light Level *size	1	1	6110	0.7064	0.4007
Phenotype*light levels *size	1	1	6110	2.9260	0.0872
Background *size	2	2	6110	4.6382	0.0097
Phenotype* background *size	2	2	6110	2.6526	0.0705
Light Level*background*size	2	2	6110	3.1778	0.0417
Remain	1	1	6110	642.4484	<.0001
Phenotype* remain	1	1	6110	0.9897	0.3199
Light levels*remain	1	1	6110	1.9257	0.1653
Phenotype*light levels*remain	1	1	6110	1.9969	0.1577
Background * remain	2	2	6110	30.5358	<.0001
Phenotype* background * remain	2	2	6110	0.1469	0.8634
Light Level*background * remain	2	2	6110	3.4783	0.0309
Size* remain	1	1	6110	67.3164	<.0001
Phenotype*size*remain	1	1	6110	12.4448	0.0004
Light Level*size*remain	1	1	6110	0.1298	0.7186
Background*size*remain	2	2	6110	8.9610	0.0001
Lightlevel*background*size*remain	2	2	6110	2.9219	0.0539

Table 4. Fixed Effects Tests for the search time model. R^2= 0.308096

Figures

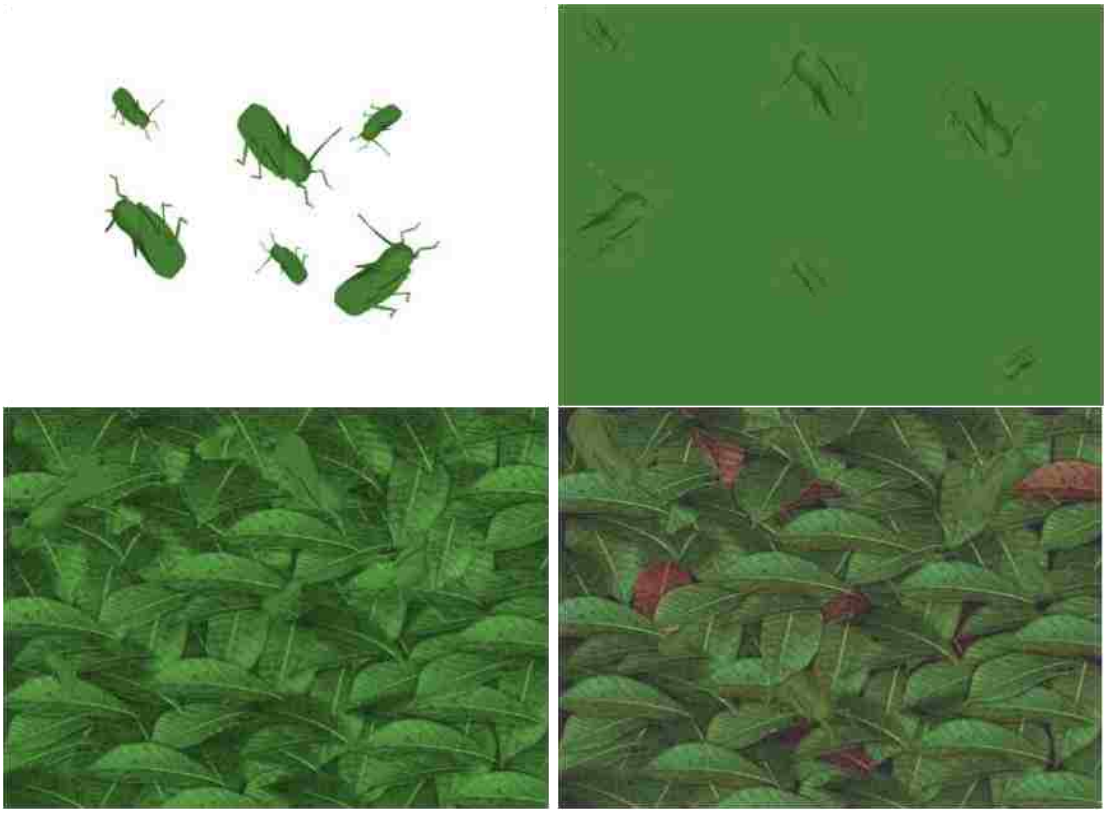

Figure 1: Example of the four experimental treatments: non-textured white background, non-textured green background, leaf-textured monochromatic green background, leaf-textured 'real' background.

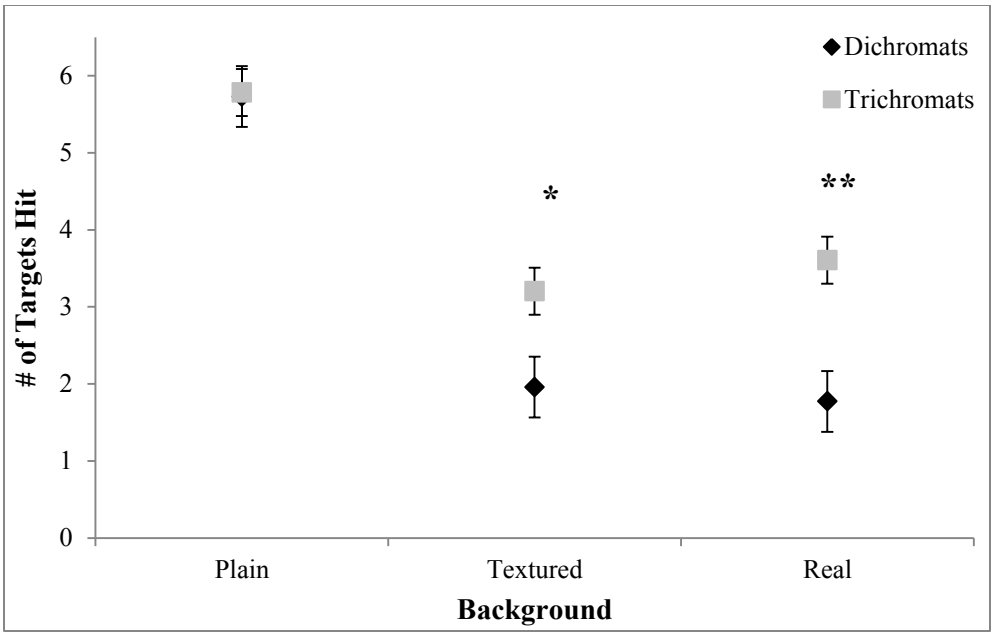

Figure 2. The average number of targets found per experiment for dichromats (n=3) and trichromats (n=6). Experimental conditions increase in complexity from left to right. Error bars represent +/- SE

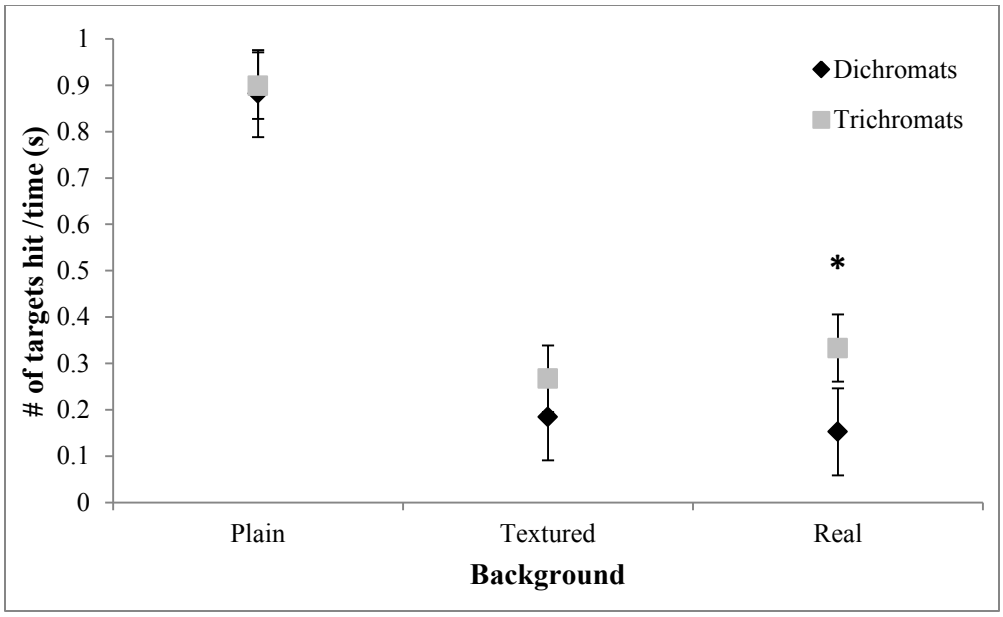

Figure 3. The average detection rate per experiment for dichromats (n=3) and trichromats(n=6). Experimental conditions increase in complexity from left to right. Error bars represent +/- SE.

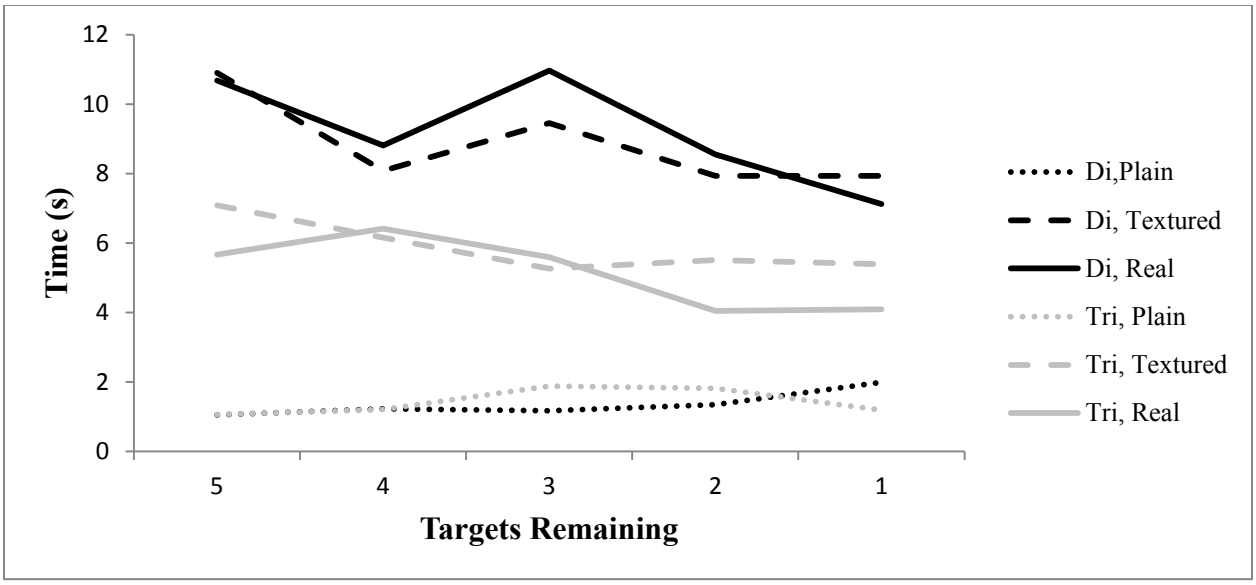

Figure 4. Search time to find the next target as a function of the number of targets remaining.

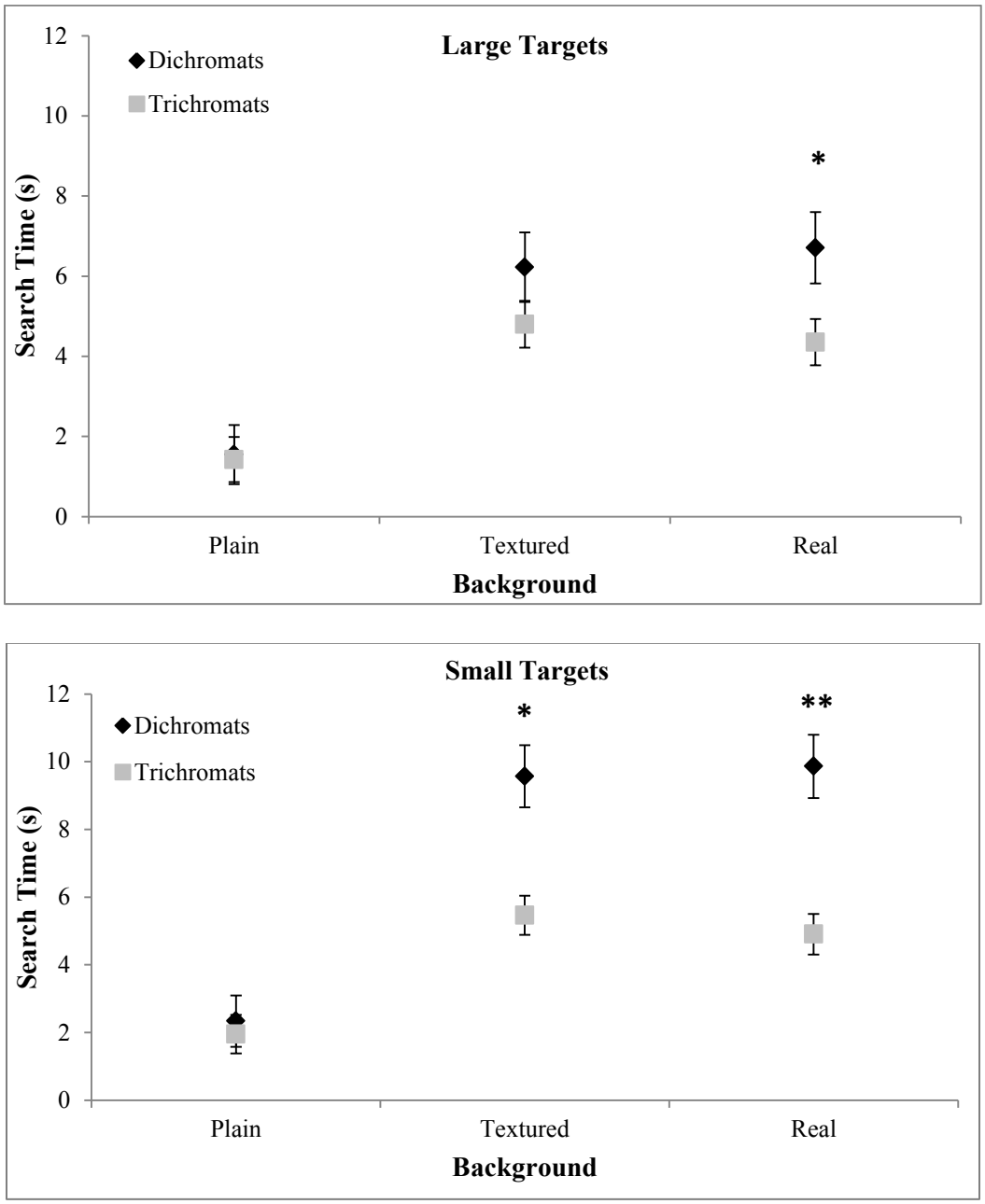

Figure 5. The search time of dichromatic and trichromatic individuals when searching for large vs. small targets embedded within monochromatic leaf-textured or "real" leaf-textured backgrounds. The number of targets remaining has been held constant at the mid-point.

Chapter 4: Use of SNP's to Determine Color Vision Genotypes from Fecal Samples of Wild Capuchin Monkeys (*Sapajus nigritus*)

A.T. Green and C.H. Janson

Introduction

Recent advances in techniques for molecular genetic analyses have improved the use of genetic material collected noninvasively from wild populations. One type of sample commonly collected from wild populations is fecal material. Both nuclear and mitochondrial DNA can be isolated from epithelial cells exfoliated from the intestinal wall during defecation (Albaugh et al. 1992). The use of fecal material for genetic analyses has been limited in part by the poor quality and low quantity of DNA extracted from feces and by the expense and difficulty of sequencing loci in many individuals.

The quality and quantity of usable material in a fecal sample depends on collection and storage methods as well as the extraction and amplification methods. The poor quality and low quantity of obtained DNA can lead to erroneous scoring of genotypes due to allelic dropout, the random amplification of only one allele at a heterozygous site during the polymerase chain reaction (PCR), and false readings due to contaminant DNA or poor PCR reaction (Taberlet et al. 1996). The success of amplification can be influenced by the fecal sample preservation method and the duration of storage before extraction. Recent advances in sequencing and genotyping technology have allowed for faster and less expensive sequencing of many samples (Kwok 2003). One such advance is the ability to detect small differences between sequences, such as single nucleotide polymorphisms (SNPs) using real-time quantitative PCR (qPCR).

The color vision polymorphism found in New World primates (platyrrhines) is a good candidate for SNP analysis using qPCR. The color vision of most NW primates is determined by alleles at the polymorphic X-linked locus coding for the opsin responsible for the middle- to long-wavelength (M/L) cone photopigment (Neitz et al. 1991). Females who are heterozygous at this locus have trichromatic vision, whereas homozygous females and all males are dichromatic. In platyrrhines, crucial non-synonymous changes at positions 180 in exon 3, and at positions 277 and 285 in exon 5 of the M/L opsin gene cause the sensitivity peak of the photopigment to vary from 530 to 565nm, with several other sites playing a minor role in the absorbance peak (Hiramatsu et al. 2004, Neitz et al. 1991, Shyue et al. 1998).

In this study we investigated the effectiveness of custom Taqman® real-time PCR probes to determine the opsin genotype of black capuchin monkeys (*Sapajus nigritus*) from four different social groups in Parque Nacional Iguazú in Argentina. More than 300 fecal samples were collected between 1995 and 2010. They were preserved in one of four ways: dried in silica gel, placed in ethanol (EtOH) and then dried in silica gel, preserved in ethanol (EtOH), and preserved in RNAlater. Our goal was to evaluate the success of SNP analysis using qPCR for differently aged and preserved fecal samples collected from wild capuchin monkeys.

Methods

Sampling

Between 1 and 4 social groups of *Sapajus nigritus* have been monitored continuously from 1991 until the present day at Parque Nacional Iguazú, Argentina. Detailed group

histories and reproductive data exist for the majority of the groups during that time period (Janson et al. 2012). While following groups, observers collected fecal samples immediately following the observed defecation and identification of the individual. The earliest samples (1995) were collected for parasite surveys and paternity determination, and were stored in 50mL vials of ethyl alcohol ranging from 70-95% concentration. Later samples were placed into silica gel, either directly after collection (1995-2003) or after a 24-hour dehydration (2004-2008) in a five-fold excess of 95% ethyl alcohol by volume (as recommended in Roeder et al. 2004). In 2009 and 2010, ATG collected samples in RNAlater solution and stored them at -20°C while in the field, then at -80°C in the lab until extraction. A typical defecation yielded about 2-3 ml of fecal material. We collected all samples with the permission of the Argentine Ministry of National Parks and relevant IACUC protocols rom Stony Brook University (2002-1218, 2003-1218, 2004-1218, 2005-1218, 2006-1218) and the University of Montana to CHJ (041-07CJDBS-120507) and ATG (024-08CJDBS-053008).

DNA extraction and genotyping

We extracted DNA using the QIAamp DNA Stool kit (Qiagen) according to the manufacturer's instructions, but with the following modifications. We left the samples overnight in the ASL buffer. For the samples in RNAlater solution, we centrifuged 2 ml of the mixture for 15 min at 7 rcf and removed the supernatant. We added 500µl of 1XTE/0.9% NaCl buffer to the "pellet" and mixed gently. We centrifuged the resulting solution for 10 more minutes at 7 rcf and removed the supernatant. The residual pellet was resuspended in 1.6 ml of ASL buffer and left overnight.

Instead of sequencing the entire exon, we used probes that targeted the three critical codons responsible for the major changes in peak light sensitivity of the X-linked opsin

photopigment protein. Table 1 shows the changes in relative sensitivity to long wavelength light from three non-synonymous changes in the M/L opsin pigment gene described above. In terms of relative spectral peaks, pigments with tyrosine at position 277 have a peak absorbance about 7 nm longer than the corresponding pigment with phenylalanine at 277; pigments with threonine at 285 have a peak about 10 nm longer than with alanine 285; pigments with serine at 180 peak about 3 nm longer than with alanine 180 (Neitz et al. 1991).

We designed custom TaqMan® assays for each amino acid site using sequences for *Sapujus* and *Saimiri* (Manusco et al. 2006, Montague 2011). We amplified regions inside Exons 3 and 5 of the M/L opsin gene separately using the following primers and reporter sequences:

SNP Name	**TYPE**	**Primer Sequence**
Exon3 site 180	Forward	5'-ATCGTGGGAGTTGCCTTCTC
	Reverse	5'-GTAGAAACCAACCTCGTCCATTCC
	TaqMan Probe 1	5'- TGGATCTGG**G**CTGCTGT-VIC
	TaqMan Probe 2	5'-CTGGATCTGG**T**CTGCTGT-FAM
Exon5 site 277	Forward	5'-CGCATGGTGGTGGTGATGAT
	Reverse	5'-TACGAAACGACGACGGTTG
	TaqMan Probe 1	5'- AGCAGACGCAG**T**ACGTCA-VIC
	TaqMan Probe 2	5'-AGACGCAG**A**ACGTCA-FAM
Exon5 site 285	Forward	5'-CGCATGGTGGTGGTGATGAT
	Reverse	5'-TACGAAACGACGACGGTTG
	TaqMan Probe 1	5'-AAGAAGG**T**GTAGGGTCC-VIC
	TaqMan Probe 2	5'-AAGG**C**GTAGGGTCC-FAM

PCR reactions contained 5 µL of DNA template, 0.28 µL of 20× assay mix, 10 µL of TaqMan® Universal Master Mix, 1 µL of 0.1 µg/µL BSA and ddH_2O to a final volume of

20 µL. The thermocycling profile consisted of an initial denaturing at 95°C for 10 min, followed by 40 cycles of denaturing at 95°C for 15 sec, annealing at 58°C for 60 sec and extension at 20°C for 30 sec. 5 cycle steps were completed after the initial 40 cycles in order to note the direction of movement on the discrimination plots. Each plate was run with a positive control for each probe and the heterozygote using known genotypes extracted from hair samples of *Sapujus*. In addition, each plate was run with five negative controls consisting of ddH_2O instead of template DNA.

The Step 1 real-time qPCR analysis software determined the genotype for each well that reached the threshold (Ct) for relative fluorescent units (RFUs). For each assay, the fluorescence emitted from the VIC-labeled probe (reporter signal 1) was plotted against the fluorescence from the FAM-labeled probe (reporter signal 2) in a scatter plot to produce an allelic discrimination plot (Figure 1).

To ensure the genotypes assigned to individuals were not erroneous due to allelic dropout or false alleles, we ran multiple samples from each individual, when available, and we ran each sample in duplicate. If there was disagreement between samples from the same individual, we ran the samples again to check the identity of the SNP. In addition, we chose 25% of the samples at random to run again to verify the previous SNP identifications. As an additional check to the SNP results, we used the 20 years of reproductive data to verify that each offspring possessed a visual genotype that matched a pattern of opsin inheritance from each reported biological parent (e.g. Table 2).

Results

We extracted and amplified 315 fecal samples. The ethanol-only samples (N=44) were from 1995; drying in silica (N= 125) was used from 1995 until 2003 while the protocol of collecting in ethanol and then drying in silica (N= 98) was used from 2004 until 2009. The RNAlater preservation protocol (N=48) was used in 2009 and 2010.

We categorized each sample as successful, dropout, and failure. A successful sample provided no ambiguity in the SNP identifications. If a sample resulted in ambiguous results for one of the SNPs, we categorized it as having allelic dropout. If the sample did not amplify or provide results for 2 or more sites, it was categorized as a failure.

Of the 315 samples, 30 (9.5%) were failures. Failures came from the ethanol only, silica only, and ethanol/silica methods; there were no failures among the most recent samples collected in RNAlater. Across all methods of preservation, 54.6% of the samples were successes; the RNAlater method had the highest success rate at 87.88%. Across all methods, 35.9% of samples showed allelic dropout at one of the three sites (Fig. 1). Amplification was significantly more successful from samples preserved using the RNAlater method than for other methods (χ^2 (6, N = 315) = 21.374, p =0.0016).

The allelic dropout rate more than doubled in RNAlater samples from 2009 compared to those collected in 2010 (Figure 3), but this difference was not signifiant (Fisher's exact test of independence χ^2 (1, N=48) = 1.571, p=0.2101). There was no consistent trend of failure or allelic dropout rate due to age of the sample for the other three methods (EtOH, EtOH/Silica, and Silica).

The spectral peaks of the M/L opsin allele photopigments were determined using the predicted estimates of peak sensitivity change for each amino acid at the three main sites (Table 1). We identified the opsin genotype for 88 individuals from 4 social groups.

Discussion

The custom Taqman® real-time PCR probes, designed this in the study, were successful in distinguishing the visual genotypes of individual capuchin monkeys (*Sapajus nigritus*) in Parque Nacional Igauzú. These probes never predicted the presence of a trichromatic male, and the results were consistent with the known family trees (Janson unpubl. data). The success of the probes depended on the quality and quantity of DNA extracted from the fecal samples, which in turn depended on the preservation method used when collecting the samples. Visual genotype could be reliably determined under all four preservation methods (EtOH, drying with Silica, EtOH/Silica, and RNAlater), but the most successful method in this study was using RNAlater. These samples were also the most recently collected fecal samples so this result is conflated with time.

We were able to reliably determine visual genotype from at least half of the samples collected 5-15 years ago using diverse inexpensive methods (70-95% ethanol; thoroughly drying the fecal pellet with silica gel; the two-step method of ethanol and then silica advocated by Roeder et al. 2004). None of these methods had demonstrably better success rates than the others for either allelic dropout or failure to amplify. For all methods, there was no consistent trend in the rates of failure or allelic dropout with year. The discrepancies among years could be due to dietary difference when collected, varying concentration of

EtOH, the dryness of the sample, or some other environmental-related influences (Frantzen 1998).

One drawback of this SNP approach is the inability to specify the multilocus genotype for each chromosome. The results using the SNP analysis showed only the heterozygosity of an individual. For instance, if an individual was S/A at position 180, Y/F at position 277, and A/T at position 285, there is no definitive way to tell if that female was SYT/AFA, SFT/AYA, SYA/AFT, or SFA/AYT. In such cases, availability of genealogical information can help to resolve some multi-locus genotypes, as the multi-locus genotype of the (dichromatic) sire is known if the sire is known, thus allowing the female's multi-locus genotype to be inferred. The inability to figure out how the SNPs distribute across the individual genes is a shared drawback with direct sequencing when using short sequences of DNA extracted from feces. If high quality DNA is available, one can perform long distance PCR to get exon 3, 4 and 5 all together in one PCR fragment along with the introns (e.g., chapter 2). Overall, the custom Taqman® probes designed in this study produced reliable color visual genotype results by SNP analysis using qPCR. SNP analysis using qPCR was a relatively cheap, quick and successful method to determine the visual phenotype of a large number of wild capuchin monkeys. This method is likely applicable for other platyrrhine species. In addition, the results of preservation method as it relates to the success of SNP analysis using qPCR may be applicable to other non-invasively collected genetic materials.

Acknowlegements: This work could not have been possible without the help from numerous people. Many thanks to Scott Miller, Darla Carvey, Erin Vogel, Erick Greene, Doug Emlen, Jack Nunberg, Maureen Neitz, Mario Di Bitetti, Maria Celia Baldovino, The Fish and

Wildlife Genomics Group in the Division of Biological Sciences at the University of Montana especially Steve Amish and Yves Hoareau, the Argentine Administration of National Parks for permission to conduct field work at Iguazú National Park and to use the accommodations at the Park's field station, as well as multiple field assistants.

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Tables

***Sapujus* M/L Opsin Allele Photopigment**	**Exon 3 Site 180 A → S +3nm**	**Exon 5 Site 277 F → Y +7nm**	**Exon 5 Site 285 A → T +10nm**
530	**Alanine**	**Phenylalanine**	**Alanine**
533	Serine	Phenylalanine	Alanine
541	**Alanine**	**Tyrosine**	**Alanine**
547	**Alanine**	**Phenylalanine**	**Threonine**
551	Serine	Tyrosine	Alanine
554	Serine	Phenylalanine	Threonine
556	**Alanine**	**Tyrosine**	**Threonine**
562	**Serine**	**Tyrosine**	**Threonine**

Table 1. Description of the three critical amino acid site changes responsible for spectral tuning of the opsin molecule. The photopigment peaks shown represent different phenotypes in terms of relative spectral peaks. The **bold** photopigment peaks have been measured by means of electroretinograms (Neitz et al. 1991). The other peaks are predicted spectral peaks using the ball park estimate of peak sensitivity change for each amino acid at the site, shown in the top row (i.e. Tyrosine (Y) at 277 has a peak about 7nm longer than the corresponding pigment with Phenylalanine (F) at 277.)

Mother ID	Offspring ID	Sex	Genotype	Genotype of likely sire
Grumpy		F	SYT/AFA	
Grumpy	Luisa	F	SYT/AFA	SYT
Grumpy	Maggi	F	SYT/SYT	SYT
Grumpy	Greta	F	SYT/AFA	SYT
Grumpy	Diego	M	SYT	SYT
Grumpy	Matteo	M	SYT	SYT
Grumpy	Pablo	M	AFA	SYT

Table 2. Example of reproductive data used to verify SNP calls.

Figures

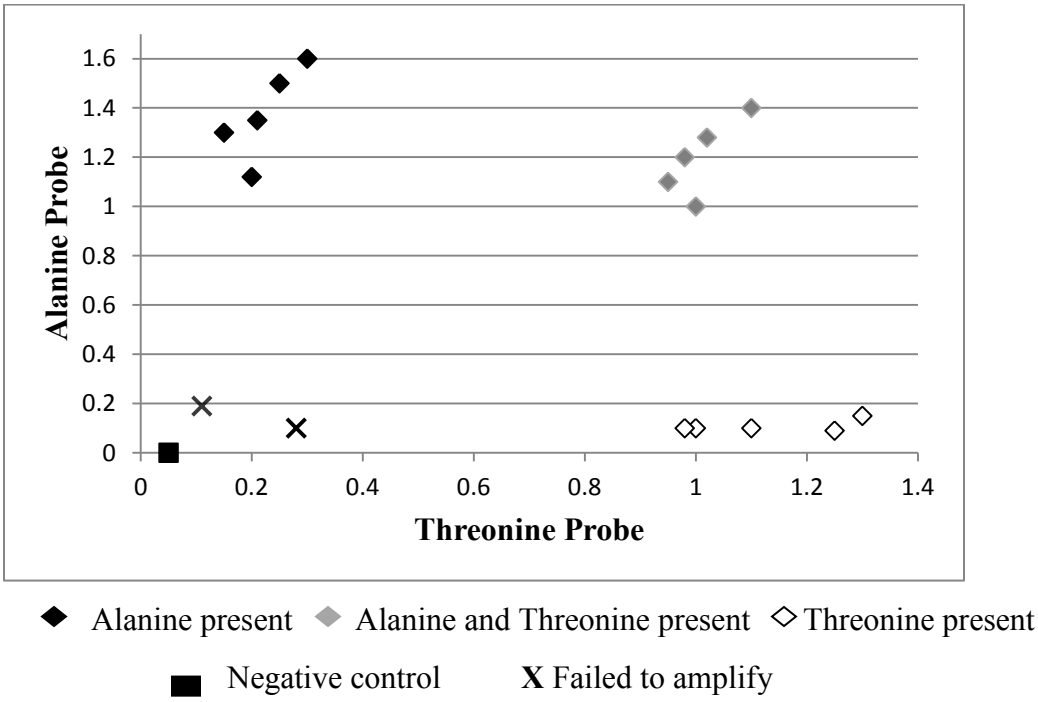

Figure 1. Example of an allelic discrimination plot for Exon 5 site 285

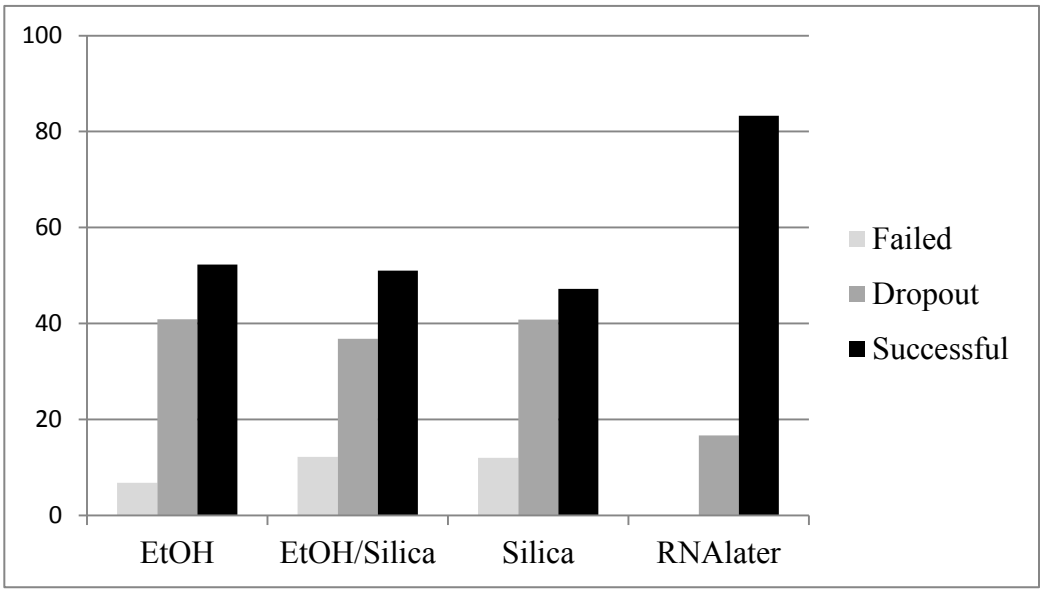

Figure 2. Rate of allelic dropout, failure, and success of four preservation methods. The rate of successful amplification at all three sites was highest for the RNAlater samples, which were collected most recently ($\chi 2$ (df=6, N=315) = 21.374, p=0.0016).

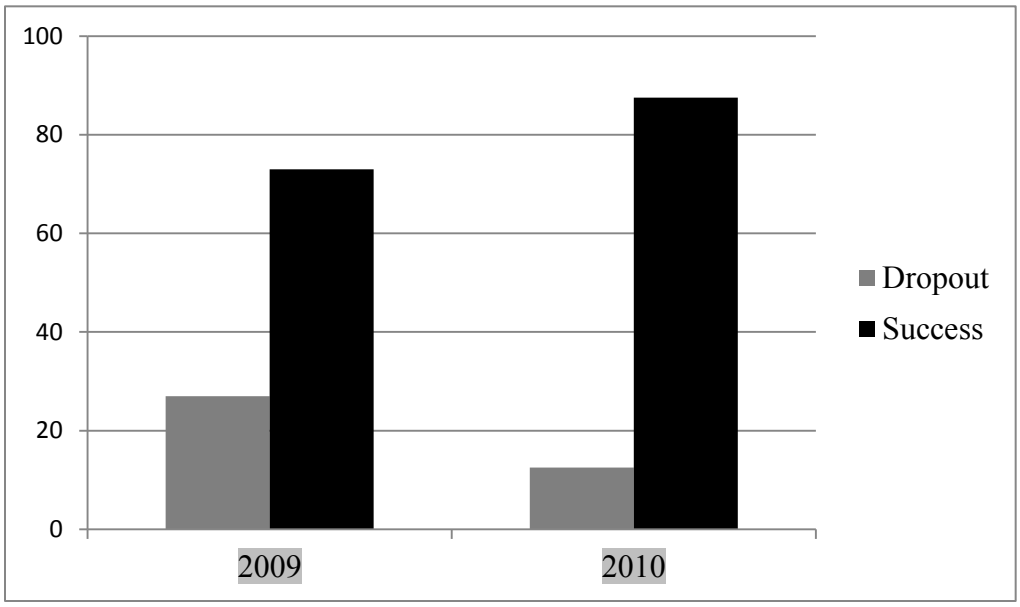

Figure 3. Allelic dropout and success rate by year using the RNAlater preservation method. The allelic dropout rate did not differ between the two years (χ^2 (df=1, N=48) = 1.571, p=0.2101).

Chapter 5: Trichromatic female capuchin monkeys are better than dichromatic females at capturing cryptic invertebrates under low light conditions.

A. T. Green and C. H. Janson

Abstract

In nearly all New World primates, 2-5 common alleles at one X-linked locus code for different opsin proteins with distinct curves of light absorption that are related to color perception. There is active debate about the mechanisms that maintain this widespread polymorphism in opsin alleles New World primates. The major hypotheses invoke 1) heterozygous advantage or 2) frequency-dependent selection. In the former, trichromatic individuals are predicted to outperform dichromatic ones consistently, whereas in the latter, dichromatic individuals are predicted to outperform trichromatic ones under some conditions. These conditions include searching for camouflaged targets at low light levels. Here we provide data on invertebrate captures by dichromatic and trichromatic capuchin monkeys (*Sapajus nigritus*) under three light levels for a wild population in Argentina. Under all light conditions, trichromatic females had higher success rates than dichromatic individuals of either sex for total invertebrate captures and for cryptic invertebrates. There were no significant differences for non-cryptic prey. In contrast to a predicted possible advantage of dichromacy under low light conditions or for camouflaged targets, the performance of dichromatic females was markedly poorer than trichromatic ones and this was especially true in the most challenging foraging tasks - searching for cryptic invertebrates under low light conditions. Our data support the heterozygous advantage hypothesis for the maintenance of polymorphic color vision.

Introduction

Uniquely among mammals, primates possess a diversity of color vision systems, ranging from ancestral dichromacy ('color-blind' vision) in nocturnal prosimians to routine trichromacy ('normal color' vision) in Old World primates. New World primates and some diurnal prosimians have an intermediate polymorphic color vision system, consisting of an invariant short (S)-wavelength sensitive opsin gene on an autosome and one middle to long-(M/L) wavelength sensitive opsin gene on the X-chromosome. In New World primates, genetic variation at the X-linked locus enables polymorphic color vision: females heterozygous at the M/L opsin locus possess trichromatic color vision, whereas homozygous females and all males possess dichromatic color vision (Jacobs and Neitz 1987, Mollon et al. 1984, Shyue et al. 1998, Tovee et al. 1994).

Patterns of DNA sequence variation, in particular heightened polymorphism in opsin gene exons compared to pseudogene and intron reference sequences, strongly indicate that this polymorphism is maintained by balancing selection (Hiwatachi et al. 2010). However, the mechanism by which selection operates on this locus remains unclear (Boissinot et al. 1998, Cropp et al. 2002, Hiwatashi et al. 2010). There are two main proposed hypotheses. One is heterozygote advantage under which trichromatic females, heterozygous at the M/L opsin locus, have an overall fitness advantage relative to homozygous dichromatic females. Because the polymorphism particularly affects discrimination among the middle and long wavelengths (Mollon et al. 1984, Osorio and Vorobeyv 1996, Regan et al. 2001, Sumner and Mollon 2000a), studies of possible trichromatic advantages have focused on foraging ability for foods with longer-wavelength reflection (colors in the green to red range). Such foraging advantages have been documented for trichromatic females in theoretical, laboratory, and

field studies (Dominy and Lucas 2001, Melin et al. 2009, Regan et al. 2001, Sumner and Mollon 2000a, 2000b). However, advantages to trichromatic females have not been consistently demonstrated in field studies (Hiramatsu et al. 2008, 2009, Melin et al. 2009, Vogel et al. 2007).

A competing set of hypotheses postulate that different visual phenotype are best adapted for different tasks and thus dichromats should outperform trichromats in some tasks or under certain conditions. (Fedigan et al. 2014, Melin et al. 2007, Mollon et al. 1984). A dichromatic advantage for target-detection tasks is predicted under low light levels or when foraging for cryptic or camouflaged prey (Morgan et al. 1992, Osorio et al. 1998, Perini et al. 2009). Because dichromats are less able to derive hue information, they are expected to be less affected by situations in which information about hue is irrelevant (cryptic/camouflaged prey) or missing (low light). Dichromatic individuals have been found in some captive and field studies to be better than trichromats at detecting camouflaged targets or when foraging in light-limited environments (Melin et al. 2007, Morgan et al. 1992, Osorio et al. 1998, Perini et al. 2009, Saito et al. 2005).

In this study, we investigated the invertebrate capture rates of dichromatic and trichromatic individuals in a population of wild black capuchins (*Sapajus nigritus*; Lynch Alfaro et al. 2012) in Argentina to determine: 1) if one phenotype had better overall success when foraging for invertebrates; and 2) if there were any combinations of prey type (cryptic vs. non-cryptic) or light environment (high, medium, or low) in which dichromatic individuals outperformed trichromatic individuals.

Methods

Study Area and Subjects

We studied a population of black capuchin monkeys in Iguazú National Park in northeastern Argentina. The study area is subtropical with seasonal variation in temperature and day length, but little variation in rainfall. Fleshy fruit and arthropod abundance is low in the winter months (June-August) and highest in the spring months (October – December) (Di Bitetti and Janson 2001). The forest in the study area and the study groups are impacted by both current and past anthropogenic disturbances from logging and tourism (Janson et al. 2012). Low visibility (distance to 95% obstruction of a checkerboard target) and light levels occur in the understory (0-5m) due to dense growth of shrubs and a common recumbent bamboo (*Chusquea ramosisima*). Both light levels and visibility increase in general with height in the vegetation, although both can be high at all heights at the edges of forest clearings. The study animals live in multi-male, multi-female groups consisting of 6 to over 40 individuals (Di Bitetti and Janson 2001, Janson et al. 2012). Black capuchins are omnivorous with a diet consisting of mainly fleshy fruit and arthropods.

Data Collection

Individuals in four study groups could be unambiguously identified by physical characteristics such as coloration patterns, size and shape of tufts, scars and behavior. Data were taken only on adult females and males. A female was considered an adult if she had reproduced, which typically meant 6 or more years old. Adult males were generally immigrants at least 7 years old. Color vision phenotypes were determined by extracting DNA from fecal samples of known individuals and using custom Taqman® real-time PCR probes to determine the opsin genotype (Green 2014). To minimize observer bias during

behavioral and foraging observations, all observers were blind to the specific opsin genotypes of each individual.

Pairs of observers followed groups from dawn to dusk for 3-25 consecutive days. Activities of paired focal individuals were recorded during synchronized 15s continuous samples taken at 1min intervals. The two observers tracked the same pair of focal animals for 10 minutes. The 10min block was considered one sample bout. Success rates were classified based on conditions during the 15s sample. The 15s sample was short enough to ensure minimal changes in light conditions due to movement. The sum of invertebrate capture attempts from samples taken under a particular light condition was divided by the summed sample time to yield the rate of invertebrate capture attempts under that condition for a given sample bout. Simultaneous sampling of two focal animals provided greater power in determining differences between color vision variants. By controlling for habitat, time of day, and group activity contexts during the sampling process, differences in foraging success or behaviors between visual phenotypes should be much more salient than when individuals of different visual phenotypes are compared across these important sources of variation. The observations were conducted on a variety of paired individuals at different times of the day, and under different environmental conditions. Because the observers did not know the visual phenotypes of females, data collection could not be efficient in targeting comparisons between distinct color-vision phenotypes. However, there was likewise no possibility of covert observer bias toward assessing capture success as higher in one female color-vision phenotype versus the other. We collected data from October 2008 until April 2010.

Ambient light conditions were measured using an Ocean Optics USB-4000 spectrophotometer with a P200-UV/VIS cable and CC-3 cosine corrector (Ocean Optics,

Dunedin, Florida, U.S.A.). Light levels were measured 4 times a year around each solstice and equinox. We measured light levels under five categories of cloud cover (0%, 25%, 50%, 75% , 100%) in four main forest types (open, bamboo dominated, above 10m and below 10m) at three forest heights (ground level, 5m and near 10m (the highest I could safely reach climbing an 8m ladder with equipment in hand)).

During foraging observations, the observers recorded date and time, forest type, canopy height, height of the focal animal, cloud cover, travel speed, group position, and light level of foraging activity. Light level was broken down into three categories: 'high': open forest or edge with no leaves or vegetation overhead; 'medium': below top of canopy with some overhead vegetation but not enough to completely block passage of sunlight; 'low': low in the vegetation with dense overhead cover which blocks much of the available sunlight. There was an approximately 10-fold decrease in irradiance from high to medium and from medium to low light levels, respectively.

Each attempt by the focal animal to capture an invertebrate was recorded as successful or unsuccessful. An 'invertebrate attempt' was defined as a lunge, grab, or manipulation of a substrate by the focal animal towards an invertebrate. Visual inspection or finger-tapping a substrate was not considered an attempt. An attempt was considered a successful capture if the invertebrate was seen in the hand or if a hand-to-mouth or chewing movement was seen following the attempt. If no hand-to-mouth movement or chewing was seen after a lunge or manipulation the attempt was deemed unsuccessful. Observers recorded the rare cases when an invertebrate was rejected after capture; these were classified as unsuccessful foraging attempts as they did not lead to ingestion. Observers described whenever possible the substrate that the invertebrate was gleaned or extracted from, as well

as the type and color of invertebrate. 'Cryptic' invertebrates were those gleaned from the exterior surface of any substrate, most often from green leaves and tree branches. These invertebrates tended to be well camouflaged against their background. 'Non-cryptic' invertebrates were those extracted from inside substrates or were larger invertebrates not notably camouflaged against their background. Examples of non-cryptic prey were termites, ants, and larvae inside dead wood or leaves, colonial invertebrates such as bees and wasps, and orb-weaving spiders in their webs. We collected focal-animal data from over 225 hours of invertebrate foraging including 5272 invertebrate capture attempts and 2622 invertebrate capture successes.

Data Analysis

The dependent variable, number of invertebrate captures per 15s sample, was an integer with relatively low mean values, for which a Poisson distribution provides the most appropriate sampling distribution. For statistical inference, we used a generalized linear mixed model fit by maximum likelihood (LME4 package in R v. 3.02: The R Foundation for Statistical Computing, 2013). After initial analysis showed the residuals of the Poisson GLMM to be overdispersed, we included a unique identifier for each observation as a random effect (following Gelman et al. 2013). Other random effects included the focal animal, the observer, and the paired experiment number. Fixed effects included the light level, and gender/visual phenotype category; duration of the sample bout was included as a covariate. Initial fitted models included all main effects and predicted interaction effects. If the initial model was significantly different from the null hypothesis (all variables have zero effect except the fitted mean), backwards stepwise selection was performed, successively eliminating the variable with the highest P value (testing against the null hypothesis that the

variable had no effect) to obtain a final model containing significant predictors. Testing male and female dichromats combined against female trichromats did not yield any results qualitatively distinct from models using all three gender/phenotype categories, so I present the latter results only.

Results

For all individuals (N=37), the average invertebrate capture attempt rate was 22.87 attempts per hour. The average capture attempt rates were higher for trichromatic females (24.75 per hour, N=14 individuals) than dichromatic females (21.65 per hour, N=11) or males (21.78 per hour, N=12) (Fig. 1, Table 1). The number of attempts decreased consistently with increasing light levels (Fig. 1, Table 1). The interaction between gender/color vision phenotype and light level on attempts per hour was not significant (Table 1).

The average individual was successful about 50% of the time with 11.33 successes per hour. The fraction of all captures that were successful was highest for trichromatic females (51.8%), followed by males (50.7%), and markedly lower for dichromatic females (44.8%). Female trichromats had more successful invertebrate captures per hour (12.83) than either female dichromats (9.69) or male dichromats (11.05) (Fig. 2, Table 2). Similar to total capture attempts, successful captures decreased consistently as light level increased (Fig. 2, Table 2). The interaction between gender/color vision phenotype and light level on successful capture attempts was not significant (Table 2).

Black capuchins capture different kinds of invertebrates when foraging. Cryptic invertebrates are generally gleaned from surfaces within the forest such as branches and

leaves while non-cryptic invertebrates are usually extracted from inside substrates such as dead wood or bamboo. We separated the invertebrate capture successes into cryptic and non-cryptic types. Success rates per hour on non-cryptic invertebrates were similar among the gender/vision phenotypes (trichromatic females = 5.93, dichromatic females = 5.93, males =6.19; Table 3), but generally decreased with increasing light level (Table 3). The interaction with gender/color vision phenotype and light level on non-cryptic invertebrate capture rates was not significant (Table 3).

Invertebrate capture rates for cryptic insets differed significantly between the three gender/phenotype categories, among light levels and due to the interaction of gender/phenotype with light levels (Fig. 4, Table 4). Trichromats had higher overall capture rates per hour on cryptic invertebrates (9.97) than did either dichromatic females (7.28) or males (8.39), and capture rates decreased under increasing light levels (Table 4). Moreover, the capture rate of trichromatic females relative to dichromatic females under low light conditions was markedly greater than expected by the main effects (significant interaction of gender/phenotype and light levels, Table 4).

Discussion

Our data demonstrate a consistent advantage in invertebrate foraging by individuals that are heterozygous for opsin alleles, with trichromatic females showing higher total capture attempts and successes per hour relative to dichromatic females or males. These differences were especially large under low light conditions. These data support the heterozygous (or trichromatic) advantage hypothesis for the maintenance of polymorphic color vision. The alternative hypotheses for the maintenance of the polymorphism at the X-

linked opsin locus postulate there are some defined conditions where dichromatic individuals should outperform trichromatic individuals, especially when foraging for cryptic prey, or when foraging under low light levels. (Caine et al. 2003, 2010, Melin et al. 2007,2008, 2010, Perini et al. 2009, Saito et al 2005). In neither of these conditions did we find that dichromats outperformed trichromats; to the contrary, trichromats were particularly successful at capturing cryptic invertebrates under low light conditions (Fig. 4, Table 4). In sum, our data allow us to reject the existing predictions about foraging performance by primate visual phenotypes under negative-frequency dependent selection. In contrast, when foraging on non-cryptic invertebrates, which often required some form of extractive foraging in which wavelength discrimination would not seem to be important, dichromatic and trichromatic capuchin females in our population performed equally well.

There were some foraging measures for which males (always dichromats) performed as well or better than trichromatic females. These cases may be confounded by gender differences in size and strength. For example, male dichromats demonstrate higher success rates foraging for non-cryptic invertebrates (Fig. 3). Because the non-cryptic invertebrates eaten by capuchins are often hidden in large, hard, or tough substrates, such as the leaf bases of Pindo palms (*Arecastrum romanizoffianum*), the larger body size and strength of males may make them more successful at foraging on this class of prey, independent of the males' visual system.

The differences in foraging success between dichromatic and trichromatic females were less marked in high or medium light conditions than under low conditions. These results are congruent with previous visual discrimination models (Osorio et al. 2004, Regan et al. 2001, Sumner and Mollon 2000), which predict that under higher light conditions

dichromats can more readily take advantage of cues other than wavelength. However, in our data, foraging attempts are less common under high light conditions for all visual phenotypes, so that a lack of statistical significance may reflect reduced sample sizes instead of, or in addition to reduced differences in relative foraging performance among gender/phenotypes. The lack of attempts while under high light conditions may reflect differences in insect abundance due to light levels or a tendency to forage for fruit instead of invertebrates when high in the forest canopy.

Recent literature on the maintenance of allelic color vision in New World monkeys calls for increased attention to mechanisms other than heterosis, citing variable results of tests of the heterozygote advantage mechanism and the existence of instances where dichromatic individuals outperform trichromatic individuals (Caine et al. 2003, 2009, Fedigan et al. 2014, Melin et al. 2007, 2010, Perini et al. 2009, Saito et al. 2005). Our studies have demonstrated clear advantages to trichromatic females (Green 2014). In our studies with captive capuchins using controlled stimuli, trichromatic females consistently exhibited superior performance relative to dichromatic females especially in low light conditions and amid complex backgrounds (Green 2014). In this wild study population of capuchin monkeys, we also found that trichromatic females exceeded dichromatic females for two components of fitness (Green 2014). The data presented here provide one possible link between phenotype and fitness for this population. It is plausible that trichromatic females achieve heavier body mass, and faster reproductive rate due to their enhanced performance in capturing protein-rich food such as invertebrates (Green 2014, Valenta and Melin 2012). The combination of all of these studies provides strong support for the heterozygote advantage hypothesis and links between phenotype, performance differences, and fitness.

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Tables

	Test statistic	Sign of effect	df	P
Full saturated model vs. without interaction	$X^2 = 8.4415$		4	0.0767
FTri vs. FDi	Z = 2.428	FTri > FDi		0.0152
FTri vs. MDi	Z = 1.237	FTri > MDi		0.215
FDi vs. MDi	Z = -1.907	FDi < MDi		0.0565
Medium vs. Low	Z = -3.861	Medium < Low		0.000113
High vs. Medium	Z = -3.429	High < Medium		0.000605
High vs. Low	Z = -6.067	High < Low		1.31e-09

Table 1. The effect of gender/visual phenotype category (MDI- male dichromat, FDI- female dichromat, FTRI- female trichromat) and light levels on total invertebrate capture attempt rates. Interaction tested was gender/phenotype category by light level. All probability levels given are comparison-wise. For details of the GLMM analysis and the main and random effects included, see Data Analysis.

	Test statistic	Sign of effect	df	P
Full saturated model vs. without interaction	$X^2 = 2.301$		4	0.6806
FTri vs. FDi	Z = 2.654	FTri > FDi		0.00795
FTri vs. MDi	Z = 0.713	FTri > MDi		0.23795
FDi vs. MDi	Z = -1.961	FDi < MDi		0.049849
Medium vs. Low	Z = -3.812	Medium < Low		0.000138
High vs. Medium	Z = -1.753	High < Medium		0.07955
High vs. Low	Z = -4.554	High < Low		5.28e-06

Table 2. The effect of gender/visual phenotype category and light levels on successful invertebrate capture rates. Interaction tested was gender/phenotype category by light level. All probability levels given are comparison-wise. For details of the GLMM analysis and the main and random effects included, see Data Analysis.

	Test statistic	Sign of effect	df	P
Full saturated model vs. without interaction	$X^2 = 2.5575$		4	0.6344
FTri vs. FDi	Z = 1.866	FTri > FDi		0.06209
FTri vs. MDi	Z = 0.366	FTri > MDi		0.35705
FDi vs. MDi	Z = -1.786	FDi < MDi		0.07407
Medium vs. Low	Z = -1.135	Medium < Low		0.25621
High vs. Medium	Z = -2.173	High < Medium		0.0298
High vs. Low	Z = -2.692	High < Low		0.00711

Table 3. The effect of gender/visual phenotype category and light levels on non-cryptic invertebrate capture success rates. Interaction tested was gender/phenotype category by light level. All probability levels given are comparison-wise. For details of the GLMM analysis and the main and random effects included, see Data Analysis.

	Test statistic	Sign of effect	df	P
Full saturated model vs. without interaction	$X^2 = 10.786$		4	0.02908
FTri:low vs. FDi:low	Z = 3.878	FTri:low > FDi:low		0.000628
FTri:low vs. MDi:low	Z = 3.927	FTri:low > MDi:low		0.000512
FDi:low vs. MDi:low	Z = -0.319	FDi:low < MDi:low		0.999
FTri:medium vs. FDi:medium	Z = 1.473	FTri:med > FDi:med		0.247
FTri:medium vs. MDi:medium	Z = 0.522	FTri:med > MDi:med		0.827
FDi:medium vs. MDi:medum	Z = -0.473	FDi:med < MDi:med		0.845
FTri:high vs. FDi:high	Z = 2.180	FTri:high > FDi:high		0.056
FTri:high vs. MDi:high	Z = 0.391	FTri:high > MDi:high		0.902
FDi:high vs. MDi:high	Z = -1.473	FDi:high < MDi:high		0.247

Table 4. The effect of gender/visual phenotype category and light levels on cryptic invertebrate capture success rates. Interaction tested was gender/phenotype category by light level. All probability levels given are comparison-wise. For details of the GLMM analysis and the main and random effects included, see Data Analysis.

Figures

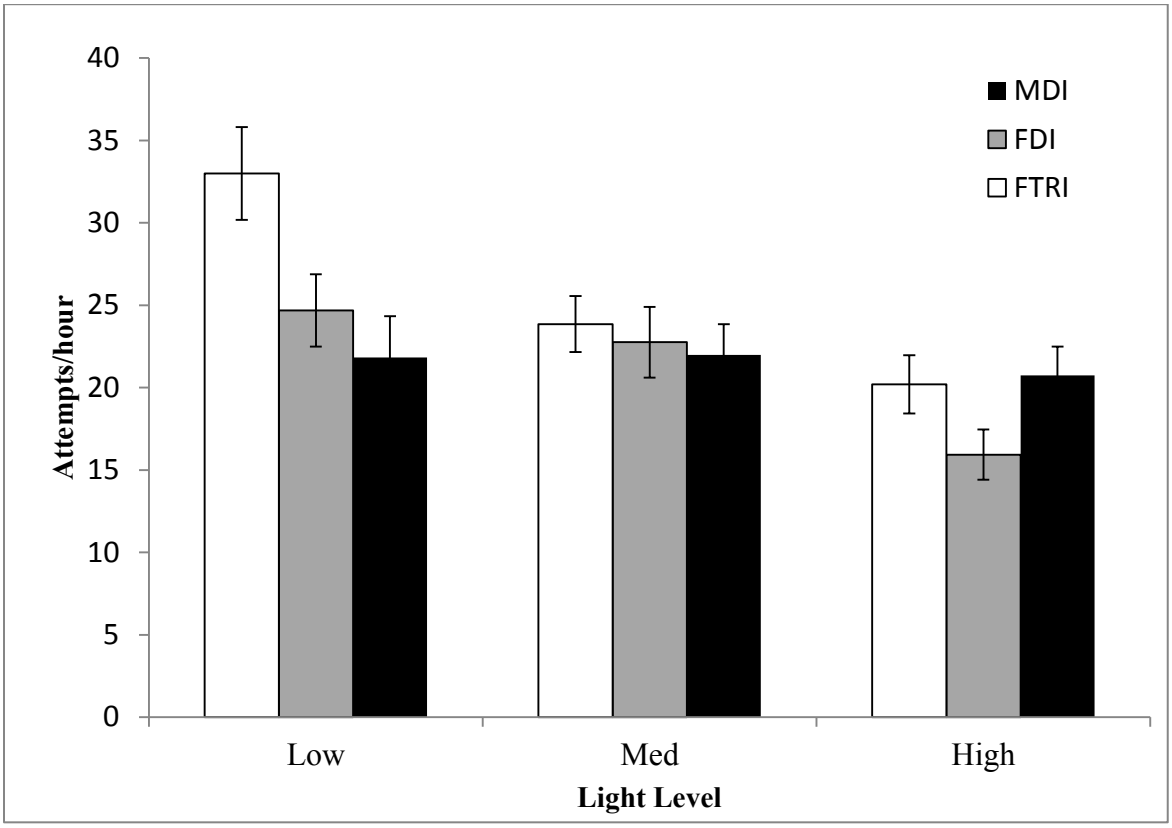

Figure 1. Total capture attempts per hour by the three gender/color vision phenotypes within each light level. (MDI- male dichromat, FDI- female dichromat, FTRI- female trichromat)

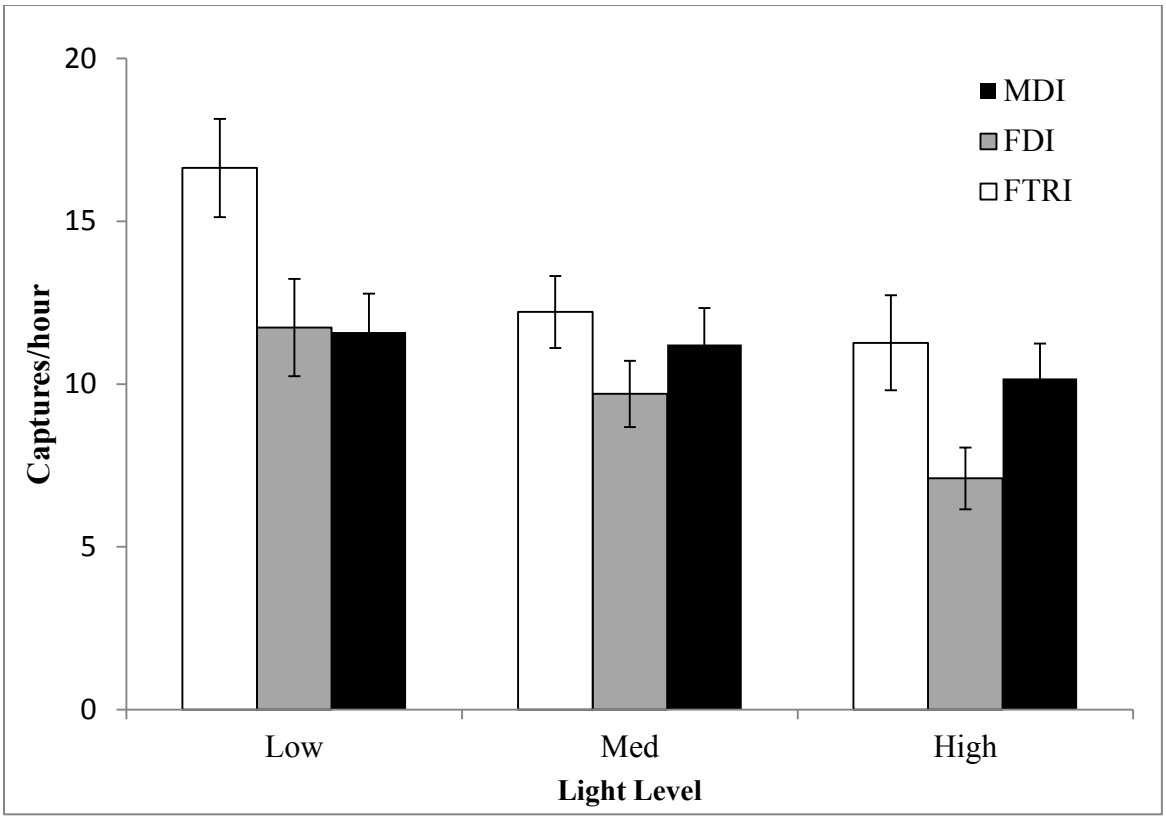

Figure 2. Successful invertebrate captures per hour by the three gender/color vision phenotypes within each light level. (MDI- male dichromat, FDI- female dichromat, FTRI- female trichromat)

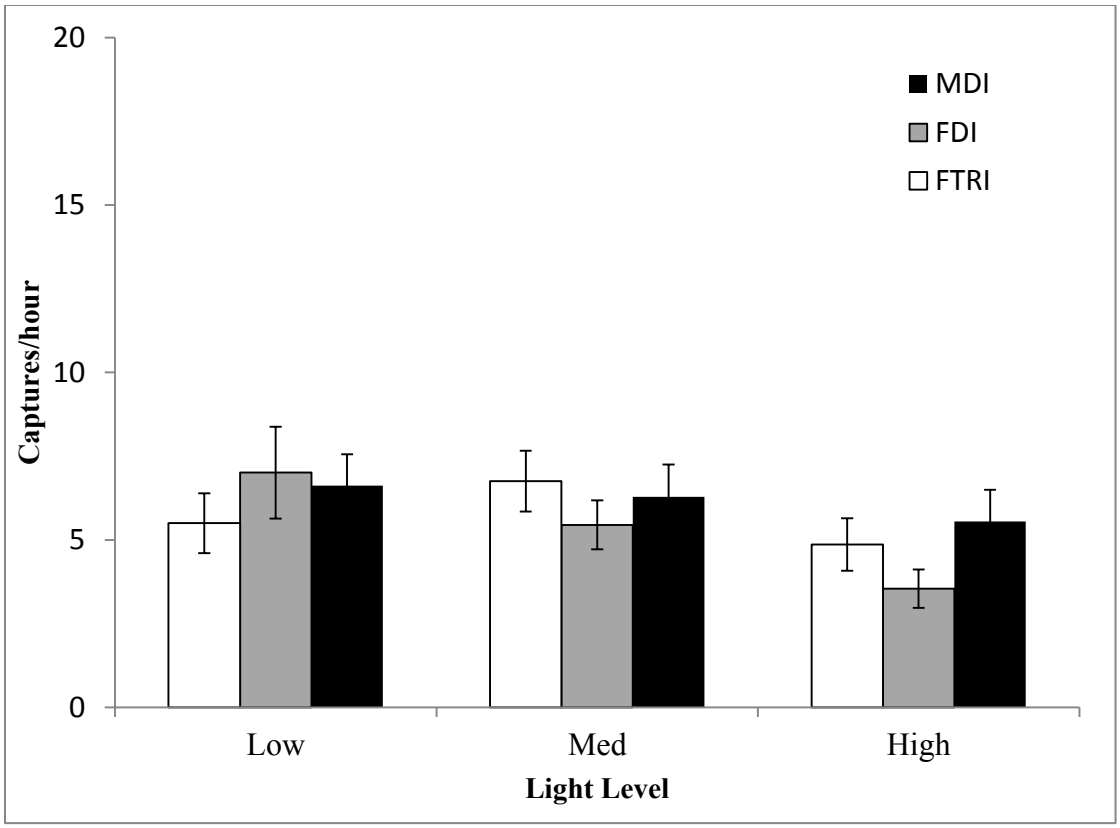

Figure 3. Successful non-cryptic invertebrate captures per hour by the three gender/color vision phenotypes within each light level. (MDI- male dichromat, FDI- female dichromat, FTRI- female trichromat)

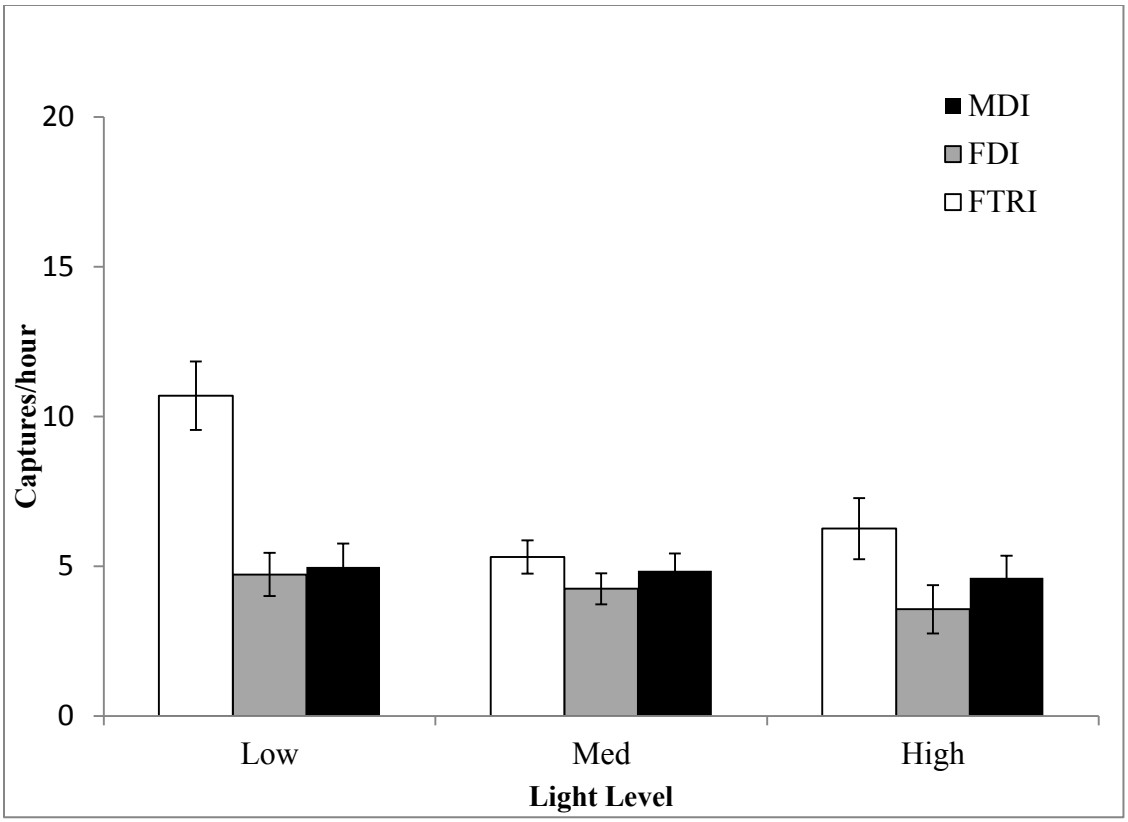

Figure 4. Successful cryptic invertebrate captures per hour by the three gender/color vision phenotypes within each light level. (MDI- male dichromat, FDI- female dichromat, FTRI- female trichromat)

Chapter 6: Fitness correlates of color vision variation in black capuchin monkeys: evidence for heterozygote advantage

A.T. Green and C.H.Janson

Abstract

We provide data on three components of fitness (body mass, reproductive rate, and survival) in a wild population of dichromatic and trichromatic monkeys (*Sapajus nigritus*) studied annually for a period of more than 20 years. For two measures, trichromatic females had higher fitness than dichromatic females. Our results support the trichromatic-advantage hypothesis for the evolution of the polymorphic color vision system characteristic of New World primates.

Introduction

The variable color vision system in New World primates provides an excellent opportunity to describe the links between genotypic and phenotypic variation, performance and fitness in natural populations. The variability in color vision arises from a well-characterized allelic polymorphism of the opsin gene on the X-chromosome that code for photoreceptor proteins, opsins, sensitive to medium (M) to long (L) wavelengths (Jacobs and Neitz 1987, Mollon et al. 1984, Shyue et al. 1998, Tovee et al. 1992). This stable polymorphism of 2-5 alleles at a single X-chromosome linked locus has been maintained for at least 14-20 million years (Boissinot et al. 1998, Hunt et al. 1998). Hemizygous males and homozygous females are functionally dichromatic (similar to red-green color blind humans) whereas heterozygous females are trichromatic (similar to normal color vision in humans). There is almost no genetic variation at the other autosomal opsin gene that codes for a retinal protein sensitive to short (S) wavelengths.

Hiwatashi et al. (2010) demonstrated strong balancing selection acting on the alleles at the sex-linked opsin gene, suggesting that the polymorphism is consistently maintained by natural selection. However, the functional causes of this balancing selection remain unclear. The **trichromatic advantage** hypothesis postulates that the polymorphism at the X-linked opsin locus is maintained by a consistent fitness advantage to heterozygous (trichromatic) individuals relative to homozygous (dichromatic) individuals. This hypothesis remains the principal explanation in the literature and is supported by theoretical modeling as well as evidence from foraging experiments conducted in the laboratory (Caine and Mundy 2000, Osorio and Vorobeyv 1996, Regan et al. 2001, Riba-Hernandez et al. 2004, 2005, Smith et al. 2003b, Sumner and Mollon 2000). Opposing this hypothesis are many behavioral observations in natural populations that have produced results that either fail to show a trichromatic advantage or show a dichromatic advantage under some conditions (Dominy et al. 2003, Fedigan et al. 2014, Melin et al. 2008, Smith et al. 2003, Vogel et al. 2007,). According to the trichromatic hypothesis, if a gene duplication event occurred at the opsin locus, fixing two functionally different alleles on one X-chromosome, it would spread rapidly in the population due to the advantages of full color vision and confer routine trichromacy to all individuals. Such a gene duplication appears to have occurred in the ancestor of Old World primates after their divergence from New World monkeys (Nathans et al. 1986), and in one genus of New World monkeys, the howler monkeys (*Alouatta*) (Dulai et al. 1999, Jacobs et al. 1996).

An alternative hypothesis postulates that selection has facilitated the persistence of dichromatic individuals in the population (Morgan et al. 1992, Osorio et al. 1998, Perini et al. 2009) suggesting that the enhanced ability of trichromats to distinguish chromatic differences

interferes with other discrimination. According to this idea, dichromatic individuals might out-perform trichromats in tasks where chromatic differences do not provide any useful information such as the perception of shape, texture, and motion detection, when foraging on cryptic foods or detecting cryptic predators, or when in low-light conditions. In support of this notion, studies conducted on natural populations have reported that dichromats may perform better than trichromats when they forage for surface-dwelling insects under low-light conditions (Melin et al. 2007, 2010).

If trichromats do not have an average fitness advantage over dichromats, then a stable polymorphism could be maintained only by **negative frequency-dependent selection**. Phenotypes that are below their equilibrium frequency in the population would be at an advantage relative to the other phenotypes. Under this mechanism, trichromats might appear to have a fitness advantage over dichromats when the former happen to be relatively uncommon, but would have a lower fitness than dichromats when trichromats are at relatively high frequency.

A third possibility, that dichromatic genotypes have an average fitness advantage over trichromats seems inherently unlikely in diurnal New World primates. If such a situation occurred, the expected genetic response would be to rapidly favor a single allele at the M/L locus, thereby eliminating the polymorphism. This response has yet to be observed in diurnal New World monkey populations. Notable levels of polymorphism are maintained in all tested New World primate species except the howler monkey, as noted above, and the nocturnal owl monkey (*Aotus trivirgatus*), in which only a single M/L allele has been maintained and the S locus has become disabled (Jacobs et al. 1996). Given this evidence and the lack of any overall advantage to dichromatic individuals in any previous study of New

World primates, one might expect to find that appropriate measures of overall fitness are greater in trichromatic than in dichromatic individuals in wild capuchins.

The link between color-vision phenotype and ecological performance has been investigated in natural populations (Bunce et al. 2011, Dominy et al. 2003, Hiramatsu et al. 2008, 2009, Melin et al. 2007, 2008, 2009, 2010, Riba-Hernandez et al. 2004, Smith et al. 2003a, 2003b, 2005, Vogel et al. 2007). One prior study, of the capuchin monkey *Cebus capucinus* (Fedigan et al. 2014), examined the link between performance and fitness, but found no difference in several fitness correlates between the color vision genotypes. Here we examine the fitness correlates of color vision variation in a different capuchin species (*Sapajus nigritus*), using three proxies for fitness: long-term reproductive success, survival, and weight. The 20+ years of demographic data on the study population in Iguazú, Argentina (Janson et al. 2012) provides a unique opportunity to relate fitness to genetic variation affecting color vision in New World primates. Our data support the trichromatic-advantage hypothesis for two fitness measures.

Methods

Study Area and Subjects

We studied a population of black capuchin monkeys (*Sapajus nigritus*) in Iguazú National Park in northeastern Argentina. The study area is subtropical with seasonality in temperature and day length, but little seasonality in rainfall. Fleshy fruit and arthropod abundance is lowest in the winter months (June-August) and highest in the spring months (October – December) (Di Bitetti and Janson 2001). The forest in the study area and the study groups are impacted by both current and past anthropogenic disturbances from logging and tourism (Janson et al. 2012). The study animals live in multi-male, multi-female groups

consisting of 6 to over 40 individuals (Di Bitetti and Janson 2001, Janson et al. 2012). Black capuchins are omnivorous with a diet that consists mainly of fleshy fruits and arthropods. Usually one male is dominant and so secures the majority of the matings and food sources (Escobar-Páramo 2000, Janson 1984). Females are philopatric and exhibit linear dominance hierarchies (Di Bitetti 1997), and adult female rank affects food intake (Janson 1985). Males typically disperse from their natal group at 5 to 9 years of age; females usually have their first birth at around 6 years of age (Janson et al. 2012). Estrous cycles begin between March-May of each year with very few females remaining receptive by August. The well-defined birth season is typically from October to January, during the peak of fruit and arthropod abundance (Di Bitteti and Janson 2001). Color vision phenotypes were determined by extracting DNA from fecal samples of known individuals and using custom Taqman® real-time PCR probes to determine the opsin genotype (Green et al. 2014).

Data Collection and Analysis

Individuals of the four main study groups were identified by physical characteristics such as coloration patterns, size and shape of tufts, scars and behavior. The population of *Sapajus nigritus* within the park has been studied since 1988 (Brown and Zunino 1990). This includes censusing twice a year since 1991 (Janson et al. 2012) as well as many behavioral and ecological studies over the years (Janson 1996, 1998, 2007, Di Bitetti and Janson 2001, Di Bitetti 2005, Baldovino and Di Bitetti 2008, Wheeler 2009, Baldovino 2010). The data included here are from four social groups derived from one original study group (Macuco), which splintered in 2005 to produce two additional groups (Rita, Gundolf), and split again in 2009 to give rise to another group (Spot).

We analyzed individual- and age-related changes in birthrate based on the presence or absence of a birth during each birth season for every female as a function of her age. Survivorship curves were calculated from the data set containing individuals born into the study groups and individuals already in the groups at the inception of study (Janson et al. 2012) and whose color vision phenotypes were known. Age was estimated for the individuals already in the group at the start of the study based on age-related changes in size, shape, and fur patterning. All individuals were followed until disappearance, death, dispersal, or the end of the 2010 study period (Janson et al. 2012).

Weights of the adult females were obtained using feeding platforms suspended from tree branches by a rope through a pulley. A scale was attached to this rope and weights of the platform were taken with a particular animal on the platform versus when no animal was on it. Three observers participated in the collection of weight data.

For statistical inference, we used a generalized linear mixed model fit by maximum likelihood. For continuous dependent variables, we used the 'Fit Model' platform in JMP (version 10.0.2 SAS Corp, 2011); for binomial dependent variables (e.g. presence or absence of a birth), we used the LME4 package in R (v. 3.02 The R Foundation for Statistical Computing, 2013). Initial fitted models included all main effects and predicted interaction effects. If the initial model was significantly different from the null hypothesis, backwards-stepwise selection was performed, successively eliminating the variable with the highest P value (testing against the null hypothesis that the variable had no effect) to obtain a final model containing only significant ($P < 0.05$) predictors. Survivorship curves were fit using the proportional hazards platform in JMP. Because of our strong uni-directional prediction

indicating higher fitness in trichromatic females, we used one-tailed tests when examining the effects of color-vision phenotype.

Results

Weights were obtained for 18 adult females (11 trichromats and 7 dichromats) between ages 6 and 27 in three of the main study groups. The average weight of these females was 2.11kg. Adult trichromatic females weighed on average 0.15kg more than adult dichromatic females (Figure 1). When controlling for age, with individual identity and observer as random effects, trichromatic females weighed significantly more than did dichromatic females ($p=0.0082$, Table 1).

The estimated birth rate for all females of known color vision phenotype was 0.527 births per female per year (N= 143 births from 31 females across 20 years; 17 females were trichromatic and 14 dichromatic). Trichromatic females had an estimated birth rate of 0.593 births per female per year, giving an approximate inter-birth interval of 20 months. Dichromatic individuals had an estimated birth rate of 0.445 births per female per year yielding an approximate inter-birth interval of 27 months (Figure 2). The difference in birth rates by color vision phenotypes was significant ($p=0.0332$) after controlling for age and prior-year birth, with individual identity as a random effect (Table 2).

The age-dependent survivorship curves for dichromatic and trichromatic females as a function of their color vision phenotype did not differ between trichromats and dichromats (proportional hazards analysis across all ages vs. color-vision phenotype, $\chi^2= 0.0134$, df=1, $p=0.7876$, Figure 3). About 89% of trichromatic females reached the age of sexual maturity compared to 94% of dichromatic females. The survivorship curves show a period of

increased mortality at the age of the first birth and nursing (6-8 years of age) followed by a period of stable survival for both dichromats and trichromats. The median predicted survival time for trichromatic females (N=18) and 26.63 and 19.74 for dichromatic females (N=18).

Discussion

Two proxies of fitness in this study lend support to the trichromatic advantage hypothesis for the maintenance of the color vision polymorphism in New World primates. Trichromatic females on average weighed 0.15kg (7.5%) more than their dichromatic counterparts. A heavier female has potentially more energy to allocate to energy-intensive reproductive events such as gestation and lactation (Clutton-Brock 1988, Gittleman and Thompson 1988), and may recover more quickly from short periods of energy deprivation (Lindstedt and Boyce 1985). These energy allocation events could, in turn, affect other fitness measures such as birth rate and survival. Indeed, trichromatic females in our study averaged 0.15 births per year more than did dichromatic females. Over an expected reproductive lifespan of about 20 years, this should equate to an increase of 3 offspring born (12 vs. 9 births, respectively).

In addition, the longest-lived trichromatic female in the study population continued to reproduce up to her last year of life at 33 years of age. The longest-lived dichromatic female stopped reproducing at 23 years of age but survived to age 27 (Janson et al. 2012). Although anecdotal, this result further supports an advantage to trichromatic individuals allowing for a higher fecundity.

Trichromatic females did not differ in survival from their dichromatic counterparts in this study. A similar negative result was found in a related species (*Cebus capucinus*) in

Costa Rica (Fedigan et al. 2014). However, it would have been essentially impossible to demonstrate statistically significant selection on survival with the sample sizes in both this study (38 females) and the Fedigan et al. 2014 study (47 females) given the small number of deaths in the younger age classes. For this reason, these negative results in the two studies are not that informative. The sample size of aged females was also limited, but three of the five females who lived past 20 years of age were trichromatic, and the longest lived female (33 years) was trichromatic. A similar trend was found in Tamarins (*Callithrix geoffroyi*) (Surridge et al. 2005).

As predicted by *a priori* functional and evolutionary arguments, trichromatic females have consistently higher values for two proxies of fitness in this population of New World monkeys. An overall fitness advantage to female dichromats is not logically plausible, as it would lead to a rapid collapse of the polymorphism by a selective sweep favoring one of the opsin alleles. However, equal mean fitness between dichromatic and trichromatic phenotypes in a population could be maintained by frequency-dependent selection (Clarke 1979, Endler 1988, Mollon et al. 1984). In a structured population such as this one, which is divided into relatively stable groups that persist across decades (Janson et al. 2012), selection favoring one phenotype is expected whenever that phenotype is locally less common than expected. Thus, the observed fitness advantages of trichromatic females in this study could have been due to negative frequency-dependent selection acting on the less common phenotype. However, trichromatic females were not less common than expected in our population. Among females of reproductive age (those most likely to compete for food and breeding success), the observed and expected fractions of trichromatic females were nearly identical: 56:8% vs. 55.7% (Green 2014). Analysis of fitness versus phenotype frequency

within individuals groups would have required more independent social groups than available in this study. Clearly, it would be desirable to expand the sample to more groups and populations.

A recently published article studying a sister species (*Cebus capucinus*: Fedigan et al. 2014) did not find evidence for a heterozygote advantage using similar fitness measures, nor did they demonstrate a fitness advantage to other phenotypes. Lack of evidence for a heterozygous advantage is not sufficient evidence to falsify that hypothesis; it is possible that the sample size was not sufficient to show an advantage to trichromats under the typically variable conditions that occur in field studies. To support the hypothesis of negative-frequency dependent selection for the maintenance of color-vision polymorphism in New World primates would require demonstrating at least on occasion a clear average fitness advantage to dichromats, a situation that has yet to be documented in any study of New World primates. In this study we demonstrated a clear fitness advantage to trichromats relative to dichromats in a natural population.

Even if the trichromatic advantage in fitness proves to be more general in New World primates, the mechanism linking phenotype to fitness is still poorly understood. Modeling of fruit-finding capability suggests that trichromatic females should have an advantage (Melin et al. 2014), while it may be that dichromatic females have an advantage in finding cryptic insects in some conditions (Melin et al. 2007). However, when tested in simulated foraging tasks in captivity, trichromatic female capuchins typically foraged faster on both conspicuous fruit-like targets and on camouflaged insect-like targets (Green 2014); only when the task was very easy did trichromats and dichromats perform equally. It is possible that food items other than conspicuous fruits provide a selective advantage to trichromatic females.

Palatable young leaves tend to be redder in hue and are used as a fallback resource during low fruit abundance by some New World primate species (Lucas et al. 2003), although not by any population of either Cebus or Sapajus yet studied. Another possibility is that trichromatic individuals are more efficient at finding cryptic fruits (Stoner et al. 2005, Melin et al. 2009), or protein-rich foods in a protein-limited environment (Valenta and Melin 2012). Further study is needed to understand the mechanisms that link color-vision phenotype and fitness in wild foraging primates.

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Tables

Source	N	DF	DFDen	F ratio	P> F
Visual Phenotype	1	1	11.01	8.0136	0.00815
Age	1	1	6.521	20.3472	0.0033

Table 1. Fixed Effects Tests for the body mass of females using a one-tailed test. R^2= 0.293272. The random effect of female identity accounted for 6.06% of the total variation. Observer accounted for 5.26% of the total variation.

Source	Estimate	SE	Z value	PR (>|z|)
Intercept	-0.900438	0.8999755	-1.001	0.31694
Prior Infant (yes)	-2.376521	0.360429	-6.594	4.29e^-11
Age Class	0.37023	0.129662	2.855	0.00430
Age Class^2	-0.01242	0.003964	-3.141	0.00168
Visual Phenotype (tri)	0.666088	0.362834	1.836	0.0332

Table2. Fixed Effects Tests for the birth rate of females using a one-tailed test. Phenotype significant in one-tailed test ($p<0.05$) when comparing models with and without visual phenotype.

Figures

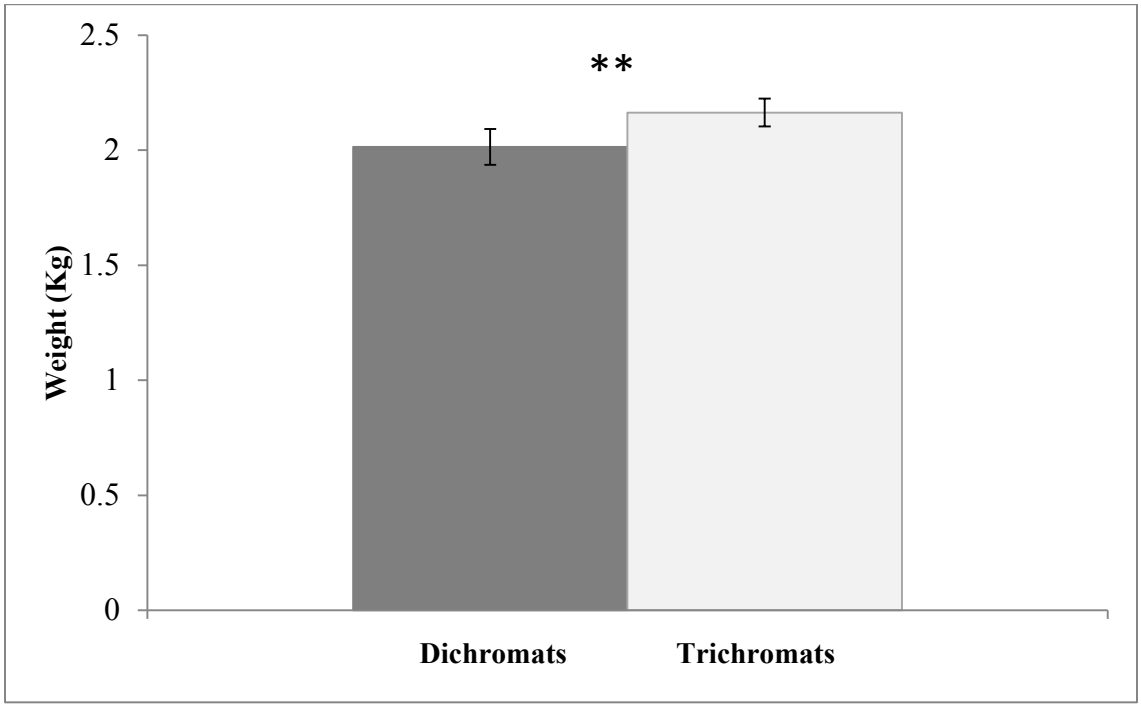

Figure 1. Weights of adult females from three study groups (Error bars are +/- standard errors).

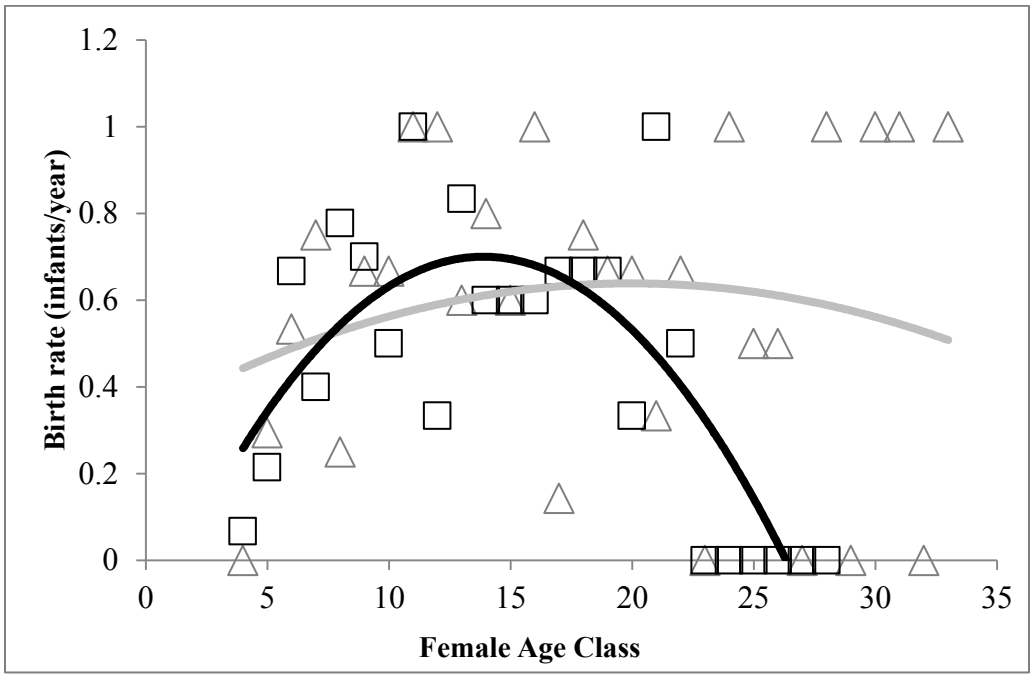

Figure 2. Birth rate plotted as a function of female age. Each symbol is the fraction of females of a given age of a given phenotype (triangles=trichromat, square=dichromat) that gave birth. The lines show the expected relationship of birth rate to age for each phenotype (grey = trichromat, black = dichromat), based on General Linear Mixed Model analysis (Table1). Controlling statistically for linear and quadratic effects of age, female identity (included as a random effect), and the presence of a surviving infant from the preceding birth season, trichromatic females had significantly higher birth rates than did dichromatic females.

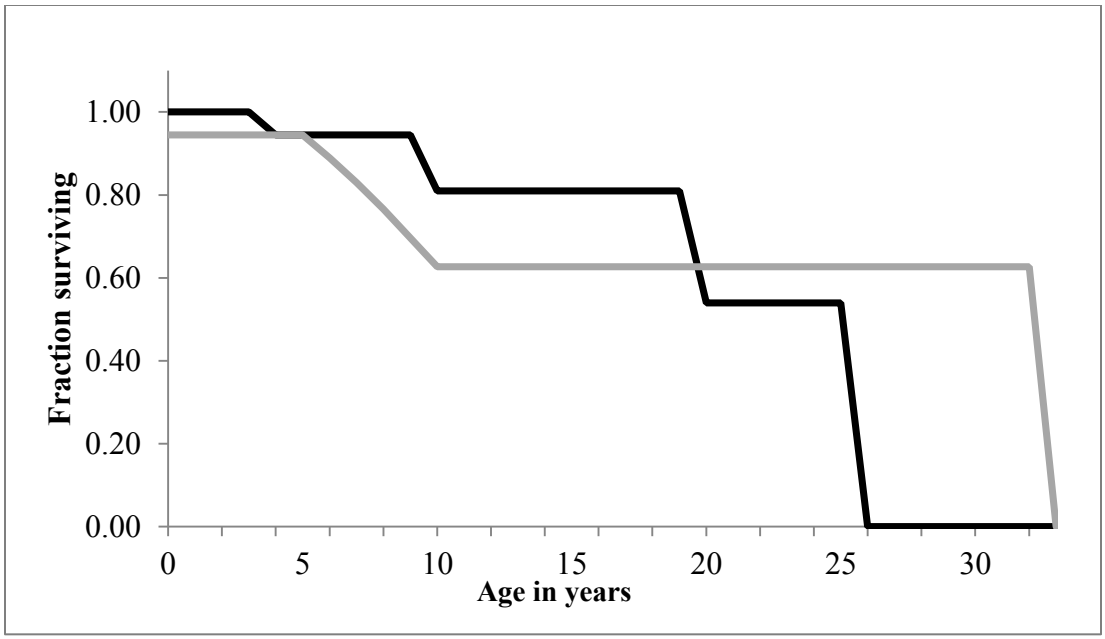

Figure 3. Age-dependent survival. Survivorship curves for all females of known color vision phenotype (grey = trichromat, black = dichromat). Because infants that died within 6 months after birth were rarely sampled genetically, the marked post-natal mortality documented across all individuals (Janson et al 2012) is not seen in this sample and the effects of color-vision phenotype at these early ages cannot be assessed.