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The Heterozygote Superiority Hypothesis for Polymorphic Color Vision Is Not Supported by Long-Term Fitness Data from Wild Neotropical Monkeys

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Abstract

The leading explanatory model for the widespread occurrence of color vision polymorphism in Neotropical primates is the heterozygote superiority hypothesis, which postulates that trichromatic individuals have a fitness advantage over other phenotypes because red-green chromatic discrimination is useful for foraging, social signaling, or predator detection. Alternative explanatory models predict that dichromatic and trichromatic phenotypes are each suited to distinct tasks. To conclusively evaluate these models, one must determine whether proposed visual advantages translate into differential fitness of trichromatic and dichromatic individuals. We tested whether color vision phenotype is a significant predictor of female fitness in a population of wild capuchins, using long-term (26 years) survival and fertility data. We found no advantage to trichromats over dichromats for three fitness measures (fertility rates, offspring survival and maternal survival). This finding suggests that a selective mechanism other than heterozygote advantage is operating to maintain the color vision polymorphism. We propose that attention be directed to field testing the alternative mechanisms of balancing selection proposed to explain opsin polymorphism: niche-divergence, frequency-dependence and mutual benefit of association. This is the first in-depth, long-term study examining the effects of color vision variation on survival and reproductive success in a naturally-occurring population of primates.

Introduction

The allelic trichromacy of long-to-middle wave sensitive (L/M) opsins in New World monkeys is a textbook example of a balanced polymorphism [1,2] and one of the few cases where the fitness consequences of variants living in natural populations are amenable to testing. Since its discovery approximately 30 years ago, this color vision polymorphism has intrigued evolutionary biologists and led to extensive debate about its utility in social signaling and finding foods, and about the nature of natural selection behind it (e.g., [3–5]). Most Neotropical primates possess variable color vision resulting from the polymorphic sex-linked L/M opsin gene and a monomorphic autosomal short-wave sensitive (S) opsin (recently reviewed by Jacobs [6]). Females heterozygous for the L/M opsin are capable of trichromatic vision, whereas the remaining homozygous females and all males are dichromatic, a condition known in humans as red-green color blindness [7].

A large and growing body of research has documented: 1) that cone-mediated color vision phenotype can be predicted in a straightforward manner from the opsin genotype, and 2) that there are clear behavioral correlates of variable color vision (e.g., [8–11]). Yet the evolutionary mechanism maintaining color vision polymorphism remains unknown. In the case of the L/M

polymorphism, we can rule out drift or random effects with a fair amount of certainty as Hiwatashi et al. [2] documented convincing support for balancing selection on the gene in question. Several hypotheses, drawn from the wider range of evolutionary mechanisms under which genetic polymorphisms are maintained via natural selection [12], have been specifically proposed to explain the maintenance of opsin polymorphisms in primates [3,4,13,14].

The most widely accredited explanatory model, the heterozygote superiority (overdominance) hypothesis, makes the assertion that individuals with trichromatic vision (females heterozygous for the L/M opsin) have a fitness advantage relative to homozygous genotypes, since red-green chromatic discrimination is useful in foraging for reddish, conspicuous fruits [15–18]; or young leaves [19]; for sociosexual signaling via pelage color [20] or patterns of blood flow [21]; or for detecting items of importance in the environment, including sympatric primate species and predators such as tropical felids [22,23,] (Figure 1). Indeed, we have shown that trichromatic females in a population of wild white-faced capuchins (*Cebus capucinus*) are more accurate in selecting ripe, reddish fruits than are males or dichromatic females [24]. However, that improved accuracy did not translate to a net increase in feeding rate, perhaps because dichromats used

behavioral compensation, had improved color perception in certain light environments or increased their reliance on other sensory modalities, such as olfaction [25–27]. Similar results for a sympatric population of polymorphic spider monkeys, documenting no difference in fruit feeding rates between dichromatic and trichromatic monkeys, are reported by Hiramatsu et al. [25,26], and by Vogel et al. [28] in their study of a neighboring population of white-faced capuchins.

The other explanatory models - niche divergence, negative frequency-dependent selection, and mutual benefit of association- share overlapping predictions contingent on dichromatic and trichromatic phenotypes each being suited to distinctive tasks [3,5]. For example, dichromats are reported to excel at breaking camouflage caused by variegated backgrounds [29,30], which assists them in detecting cryptic predators and prey. Additionally, studies of New World monkeys have shown that dichromatic females and males are more efficient than are trichromats at detecting and capturing camouflaged objects (e.g., insects) especially under low ambient light conditions [31–34]. Divergent abilities due to vision phenotype could therefore allow individuals to specialize on different foods and decrease intragroup feeding competition. Monkeys with rare phenotypes might be especially favored if they experience the least competition for their preferred food type. As social organisms, dichromatic monkeys may also benefit from co-residing with trichromats who lead the group to conspicuously colored fruit trees [18] or who first spot yellowish felids [23]. In turn, trichromats may benefit from capturing camouflaged insects flushed by dichromatic group members [35], or by hearing their alarm calls given to cryptic predatory snakes.

As noted by Cropp et al. [36], it is premature to choose among these adaptationist explanations without studies that examine fitness variability among individuals of different phenotypes. Similarly, Surridge et al. [4] have argued that researchers need to conduct studies to determine whether the proposed visual advantages experienced by trichromats or dichromats actually translate into increased survival and reproductive success. The goal of our paper is to provide such a comparative report on fitness in the trichromatic and dichromatic females of a wild population of Costa Rican white-faced capuchins. We have previously documented a tri-allelic L/M opsin gene polymorphism in this population, individual members of which exhibit one of three dichromatic phenotypes that are traditionally named after the peak spectral sensitivity of their L/M photopigments: Red (561 nm), Yellow (543 nm) or Green (532 nm), or one of three trichromatic phenotypes (Green/Red, Green/Yellow, Red/Yellow) [2,37]. We predict that if the L/M polymorphism is maintained via heterozygote superiority, then trichromatic females should have better survival or greater reproductive success than do dichromatic females. Alternatively, if overdominance is not operating and the polymorphism is maintained by an alternate mechanism, then we predict that trichromatic and dichromatic females will have similar overall fitness.

Research on differential reproductive success in wild primates has examined many possible predictors and come to often contradictory results as to the effects of dominance rank, maternal age, infant sex, resource availability and group size on variable fitness of individuals, (see review by Pusey [38]). Although age and dominance rank of the mother are often good predictors of reproductive success across many primate species, this is not true in all primate populations and our previous analyses have shown that both maternal age and rank fail to predict female reproductive success in our study animals [39], perhaps because of the confounding effects of frequent aggressive invasions of groups and associated infanticide by immigrating capuchin males [40,41]. However, the production and survival rates of a female capuchin's infants are well predicted by the number of matrilineal kin and by the adult male: female ratio in her group (higher ratio of resident adult males predicts greater female reproductive success), as well as

Figure 1. How female capuchins see the world. Color vision phenotype affects perception of relevant objects in the natural environment, including sympatric primates (top row, *Ateles geoffroyi*, photo credit F. Campos), ripe dietary fruits (middle row, *Ficus ovalis*, photo credit A. Melin) and predators (bottom row, *Puma concolor*, photo credit N. Parr). Renditions of dichromatic vision (left column of images) were generated via a computer program customized to simulate primate color vision [55] and were based on the most common dichromatic capuchin phenotype (L/M allele with peak sensitivity of 561 nm).
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by the availability of resources and whether or not her previous infant survived past one year of age [42].

In the present study, we build on previous work examining differential reproductive success in primates by testing whether color vision phenotype is a significant predictor of female fitness in wild white-faced capuchin monkeys. We also build on previous tests of the overdominance hypothesis that found no trichromat advantages in fruit feeding rates [24–26,28] by here providing a conclusive test of whether proposed trichromatic visual advantages translate to enhanced fitness in our study animals.

Methods

Ethics Statement

The research reported in this study adhered to protocols approved by the Canada Council for Animal Care through the University of Calgary's Life and Environmental Animal Care Committee (LESACC, protocol numbers: AC11-0082, BIO82-03, BI 2008-03, BI 2005-07, BI 2002-08), and the University of Alberta's Biosciences Animal Care Committee (protocol numbers: 610151, 151804, 319104). Our research also adhered to the laws of Costa Rica and was conducted with permission from the administrators of the Área de Conservación Guanacaste and the National Park Service of Costa Rica (ACG-PI-026-2013).

We have submitted research protocols on annual applications to the University of Calgary's and the University of Alberta's Animal Care Committees and they have approved all the protocols that we used to collect the data analyzed in this study (i.e., observational data collection on behavior, births, naturally-occurring mortality and collection of fecal samples). The analyses in this paper are based on 26 years of life history data (births and naturally-occurring deaths) as well as color vision genetics data extracted from fecal samples. Fecal samples are collected non-invasively from the ground below the trees or low-lying vegetation following the defecation of an individual monkey. The monkeys of Santa Rosa are a naturally-occurring population, free-living in a national park and no monkeys were handled or interacted with during this study.

Study Species and Site

White-faced capuchins (*Cebus capucinus*) occur in Central and South America from Honduras at the northern edge of their range down to the northwestern corners of Ecuador and Colombia in the south. They are arboreal omnivores that can survive in a variety of habitats and consume an eclectic diet of fruit, flowers, pith, invertebrates and small vertebrates. They live in multi-male, multi-female groups made up of natal females, immigrant males and their offspring. Park-wide censuses of all the capuchin groups in Santa Rosa indicate an average group size of 17 and an overall adult sex ratio approaching 1:1 [43]. Males disperse from their natal group around the age of 4.5 and continue to move between groups approximately every four years, sometimes alone and sometimes in parallel with their male kin (e.g., siblings, cousins) [44,45]. Males sometimes immigrate into groups peacefully when they are not fully grown or when the group has been vacated by the resident males, but most adult and subadult males enter groups by force in coalitions that challenge and drive the prior resident males from the group [41]. During and soon after these aggressive take-overs, many group members are injured and infants often die. Infanticide is the major source of mortality in the first year of life [40].

Females on the other hand, remain in their natal groups, except for a few individuals (12%) who occasionally leave with the former resident alpha male when he departs the group, and together they join a neighboring group [46]. Females first give birth at the mean age of 6.5 years and there is little variation around age at first birth (5.9 to 7.3 years; 68% of females first give birth in their sixth year of life). Subsequent to first parturition, females typically give birth every two years thereafter; unless the first infant dies, in which case the interbirth interval averages 1 year. Female rank does not affect interbirth interval length and dominance rank is not a life-long characteristic of individual monkeys [39].

The site of our study, Santa Rosa National Park in Costa Rica, was established in 1971 and encompassed 108 km² of tropical dry forest before it was amalgamated in the late 1980s with small neighboring parks and ranchlands into the mega-park, Área de Conservación Guanacaste (ACG). The core of ACG remains as Sector Santa Rosa, which is the location of the capuchin groups that we have studied since 1983. Detailed description of this site and species as well as life history and census data on the Santa Rosa capuchins can be found in Fedigan and Jack [43].

Study Sample and Determination of Color Vision Phenotype

The data analyzed herein come from four closely-studied and contiguous social groups of Santa Rosa capuchins (CP, LV, EX and GN) on which we have up to 26 years of life history data. These groups have been continuously monitored since their start-of-study dates (CP: 1983–2012; LV: 1990–2012; GN & EX: 2007–2012) with the exception of seven gaps in CP and LV data collection lasting 2–6 months each (most gaps were two months in length). Birth dates of 15 infants born during these gaps were estimated based on their morphology when we first encountered them and our extensive experience with approximately 140 infants that we have observed closely since the exact day or week of their birth. Death dates of monkeys that disappeared during the gaps were assigned as the mid-point of the period. It is possible that we missed some infants that were born and died during these short periods when groups were not monitored. When new researchers joined our team, they were trained in the field until they could reliably recognize all individuals, ensuring continuity and accuracy of identity and age assignments. Following this method, we have collected data on well-known monkeys over their complete lifetimes and up to five generations of females have been observed in CP group.

Thus far, we have tracked the survival and reproductive lives of 101 females living in these four adjacent groups. Of these, we determined the color vision phenotype for 49 females (21 dichromats, 28 trichromats). Forty-eight of these 49 color-typed individuals lived beyond two years of age and were included in the longevity (post-weaning survival) analysis. Thirty-seven females produced infants (17 dichromatic and 20 trichromatic mothers) and were included in the reproductive analyses (Table 1). Among the trichromatic mothers, the Green/Red phenotype was most common and among the dichromatic mothers, the Red phenotype was most common (Table 1).

We performed color vision genotyping from fecal DNA collected non-invasively. Multiple (2–5) fecal samples were collected from all individuals in each social group. Approximately 1 gm of feces was stored at ambient temperature in 5 ml of ASL buffer (QIAamp DNA Stool Mini Kit; Qiagen), pre-dispensed into sterile 15 ml plastic vials. Fecal DNA was isolated using the QIAamp DNA stool mini kit (Qiagen Inc.) in a biological safety cabinet. We sequenced the L/M opsin genes of each individual from a minimum of two different fecal samples, requiring two identical results to assign a color vision genotype. Entire gene sequences of the three alleles of *Cebus capucinus* were registered in GenBank in 2005 under accession numbers AB193773 (P561

Table 1. Female Capuchin Color Vision Phenotypes, Survivorship, and Production of Infants. M-L pigment sensitivity describes the peak spectral sensitivity (λ_{max}) of the constituent middle-to-long wavelength sensitive photopigment(s) (red = 561 nm, yellow = 543 nm, green = 532 nm).

Animal ID	Color Vision Phenotype	M-L Pigment sensitivity(ies)	Age at Departure (years)	Depart Type	# of Offspring	mean IBI[a] (years)	mean IBI[b] (years)
BAL	Dichromat	Red	9.55	End of Study	1		
CHA	Dichromat	Red	8.05	End of Study	1		
ED	Dichromat	Red	12.52	End of Study	5	2.34	0.93
KIA	Dichromat	Red	10.56	End of Study	2	2.88	
LIM	Dichromat	Red	22.05	Death	8	2.21	0.67
NEM	Dichromat	Red	8.69	End of Study	1		
NYL	Dichromat	Red	11.11	Death	4	1.83	1.16
PIC	Dichromat	Yellow	9.60	End of Study	2	2.06	
PUM	Dichromat	Red	10.59	End of Study	3	1.95	
SAR	Dichromat	Red	11.88	End of Study	3	1.86	
SER	Dichromat	Red	23.44	End of Study	7	2.26	
SHA	Dichromat	Red	9.71	End of Study	2	3.04	
SHE	Dichromat	Red	3.58	Death	0		
SIM	Dichromat	Red	14.29	End of Study	4	2.06	
TIM	Dichromat	Red	16.51	End of Study	4	1.93	
ZAZ	Dichromat	Red	13.80	End of Study	3	2.56	
ROS	Dichromat	Red	19.37	Death	2	1.49	
RIT	Dichromat	Red	12.88	End of Study	3	1.96	
FAW	Dichromat	Yellow	1.21	Death	0		
QUI	Dichromat	Red	5.55	End of Study	0		
GAI	Dichromat	Yellow	4.36	End of Study	0		
ABU	Trichromat	Green/Red	7.57	End of Study	0		
BLA	Trichromat	Green/Yellow	26.75	Death	10	2.05	1.02
CHU	Trichromat	Yellow/Red	13.30	End of Study	5	2.24	0.90
DOS	Trichromat	Green/Red	20.15	Death	8	1.77	1.01
KAT	Trichromat	Yellow/Red	23.19	Death	11	1.83	0.88
MAY	Trichromat	Yellow/Red	6.20	Death	1		
ORE	Trichromat	Green/Red	7.61	End of Study	1		
SAL	Trichromat	Green/Yellow	16.72	End of Study	6	1.83	
VEL	Trichromat	Green/Red	6.22	Death	0		
BEA	Trichromat	Green/Red	6.69	End of Study	0		
ARI	Trichromat	Green/Red	6.45	End of Study	0		
PAN	Trichromat	Green/Red	2.35	Death	0		
MIN	Trichromat	Green/Yellow	22.88[c]	End of Study	3	2.27	1.69
MAX	Trichromat	Yellow/Red	19.81[c]	Death	3	1.80	
LUN	Trichromat	Green/Yellow	22.88[c]	End of Study	3	2.05	1.84
FLE	Trichromat	Green/Red	15.88[c]	End of Study	3	2.08	
LIL	Trichromat	Yellow/Red	15.88[c]	End of Study	4	2.67	0.99
PET	Trichromat	Green/Red	13.88[c]	End of Study	3	2.61	
MRS	Trichromat	Green/Red	18.88[c]	End of Study	6	2.21	0.98
PAD	Trichromat	Green/Yellow	9.88	End of Study	1		
CHO	Trichromat	Green/Red	9.35	Death	2		0.74
ATH	Trichromat	Yellow/Red	12.14[c]	Death	3	3.16	
ELE	Trichromat	Green/Yellow	13.88[c]	End of Study	3	1.95	
CAL	Trichromat	Green/Red	11.88[c]	End of Study	5	1.65	0.78
HEL	Trichromat	Yellow/Red	9.88	End of Study	3	2.05	1.00
CRE	Trichromat	Green/Red	2.77	Death	0		

Table 1. Cont.

Animal ID	Color Vision Phenotype	M-L Pigment sensitivity(ies)	Age at Departure (years)	Depart Type	# of Offspring	mean IBI[a] (years)	mean IBI[b] (years)
THY	Trichromat	Green/Red	4.47	End of Study	0		
CAS	Trichromat	Green/Red	2.97	Death	0		

a = Mean of uncensored (complete) IBIs when the first infant in the interval lived ≥ one year of age.
b = Mean of uncensored (complete) IBIs when the first infant in the interval died<one year of age.
c = entered study as an adult or subadult, age estimate based on morphological features at first sighting in 2007.
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allele), AB193778 (P543 allele) and AB193784 (P532 allele). Amino acid residues at the three critical tuning sites (exon 3, site 180; exon 5 sites 277 and 285) were determined to assign the color vision genotype [26]. We minimized the chances of allelic dropout by requiring that at least one fecal sample from each female contained no less than 200 pg of genomic DNA. Further details, including our PCR and sequencing protocols, are described in previous publications [24,26].

Measures of Fitness and Reproductive Success

The rate of infant production (fertility) and survival of those infants, and longevity of the mother herself, are three important components of female fitness [38,47,48]. We address these three variables in turn.

In iteroparous organisms that habitually give birth to one infant at a time (most primates), fertility rates depend on the length of time between parturition events, i.e., the interbirth interval, or IBI [49]. We calculated the intervals between live births as our measure of fertility rate. IBIs are commonly used in primatology as a proxy for the number of infants born per female in a given time interval [38,50], and since our data on births were unavoidably constrained by the arbitrary start and stop dates of our study, it was more appropriate to use IBIs rather than number of infants born as our measure of female fertility. We included in our analyses intervals that were right censored by the stop date of our study or death/departure of the mother, since their exclusion may result in systematic bias toward shorter intervals [51]. Furthermore, our previous finding that death of an infant prior to 1 year of age shortens the length of the interbirth interval [39] led us to distinguish between intervals in which the first infant died prior to the age of 1 year and those in which the first infant survived. By separating out IBIs in which the first infant died prematurely, we were also able to remove any effects of early infant death/infanticide on IBI length.

To be reproductively successful, a female primate needs not only to produce infants but also to experience high rates of survival in her offspring and to live a comparatively long life herself. We examined the survival of a female's infants from their births to two years of age. We used age two as the cut-off point for calculating offspring survival on the assumption that prior to this age, an immature monkey's survival would be primarily a function of their mother's rather than their own color vision phenotype. This is because in the first year or two of life, prior to weaning, a young capuchin depends on its mother for milk and because the mother's ecological fitness and health determine the availability of her milk and the amount of care (e.g., transportation) and protection she can provide to the infant.

Our second measure of survival was that of the mother herself (longevity) which we calculated from two years of age (i.e. post-weaning) until her death or the end of our study. Sometimes we find the cadaver of a deceased study animal on the forest floor, or

we observe them to be wounded or ill before they disappear, in which case we record them as dead. Females seldom disperse (~12% of females have emigrated out of, or immigrated into our study groups) and in the few cases where females have dispersed from our study groups, we have tracked them to a neighboring group. Therefore, we assume that any cases of post-weaning female disappearances are deaths.

For both IBIs and the survival of infants, we accounted for potential autocorrelations in the fitness events experienced by each particular mother by introducing a random effect of the identity of the mother in the analyses. As noted by Jones et al. [49], introducing the random effect of the mother's identity also provides an indirect measure of "phenotypic quality" or "frailty."

Statistical Analyses

To assess the pace of infant production, we used mixed effects Cox regressions (coxme and coxph procedures) [52] in R [53] and analyzed the length of 139 interbirth intervals (IBIs, Table 1). There were a total of 101 complete/uncensored and 38 censored intervals for 37 mothers of known visual phenotypes. The model included IBI as the dependent variable, a fixed effect of the mother's color vision phenotype (dichromatic or trichromatic), a fixed effect of whether the first infant in the interval died prior to age 1, and a random effect of the identity of the adult female. Censored values included cases where the female was still alive at the end of the study but had not yet given birth to another infant and cases where a female had died. Thus, if a female had N births, there were N values for IBI, the first being the interval between births 1 and 2, and the last being the censored interval between last birth and death/end of study.

To determine the effect of the mother's color vision type and of the mother's identity on the survival of her infants, we used mixed effects Cox regressions (combining coxme and coxph procedures) in R to analyze the survival of infants from birth to age 2. We included all 139 infants born to 37 females with known color vision phenotypes (Table 1). The model included age of the infant at death or at the end of the study if the infant was still alive but less than 2 years of age, as well as a fixed effect of the mother's color vision type and a random effect for the identity of the adult female.

To examine the longevity of trichromatic versus dichromatic females, we used a Cox proportional hazard regression (the coxph procedure in R) to analyze the survival of individual females from the age of two years on. There were a total of 48 females included in this analysis for which we know their color vision phenotype and that they survived beyond two years of age (Table 1). The model included a survival function for the females as a function of being trichromatic or dichromatic. The age at death was right-censored for those individuals still alive at the end of the study. The age of entry into the study was left censored if the individual did not enter the study at birth (in which case we estimated her age, based on age-related morphological features such as brow and nipple

length, and based on 26 years of observing the aging process in females of known age).

Because the advantage proposed to exist for trichromats is hypothesized to be particularly strong for those that have maximum sensitivity near the green or red end of the spectrum, we repeated all three analyses (IBI, offspring survival, maternal survival), using only reproductive data from Green/Red trichromats and comparing them to dichromats.

Results

Interbirth Interval Duration in Trichromatic versus Dichromatic Females

We examined the length of interbirth intervals (IBIs; N = 139) as a function of the effect of: (1) the mother's color vision phenotype; (2) the death of the first infant in the interval at <1 year of age; and (3) the random effect of the mother's identity. The interbirth intervals of trichromatic versus dichromatic females did not differ significantly ($\chi^2 = 0.445$, df = 1, p = 0.504, Figure 2). For IBIs where the first infant in the interval lived at least one year, the fitted median IBI was 2.05 years (N = 56) for trichromatic females and 2.19 years (N = 45) for dichromatic females.

In accordance with a previous study [39], we found that interbirth intervals in which the first infant died before age 1 were significantly shorter than intervals in which the first infant survived ($\chi^2 = 59.5$, df = 1, P<0.001) and this was true for both trichromats and dichromats (Figure 2). For IBIs where the first infant in the interval died before age 1, the fitted median IBI was 1.01 years (N = 28) for trichromatic females and 1.13 years (N = 10) for dichromatic females.

Finally, we found that the individual identity of the mother (i.e., the random effect of the mother independent of her vision phenotype) contributed significantly to the explanatory power of the model ($\chi^2 = 4.460$, df = 1, p = 0.035).

Survival of Infants Born to Trichromatic versus Dichromatic Mothers

We assessed the survival of infants (N = 139) from birth to the age of two years, the typical timing of weaning, as a fixed effect of the mother's color vision phenotype and the random effect of the mother's identity. Infants of trichromatic mothers did not live longer (up to two years) than those born to dichromatic mothers ($\chi^2 = 1.49$, df = 1, p = 0.221), nor did the identity of the mother contribute to explaining variation in the probability of the offspring surviving to the age of two ($\chi^2 = 0.005$, df = 1, p = 0.940, Figure 3). For trichromatic females, 61.9% of their offspring survived to age 2, whereas for dichromatic females, 79.6% of their offspring survived to age 2.

Survival of Trichromatic versus Dichromatic Females Post Weaning

When we examined the survival of females past the age of 2 years as a function of their color vision phenotype we found that survival did not differ between trichromats and dichromats ($\chi^2 = 0.91$, df = 1, p = 0.339, Figure 4). The median predicted survival time for dichromatic females was 22.1 years (N = 20) and 19.8 years for trichromats (N = 28). Additionally, if we consider only those females who actually died before the end of our study, we can see from Table 1 that they lived between 1.21 and 26.75 years. The mean age of survival for the trichromatic females who died before the end of the study (n = 11) was 11.99 years and the mean age of survival for dichromatic females who died (N = 5) was 11.46 years.

Comparison of Green/Red Trichromats to Dichromats for Interbirth Intervals, Offspring Survival and Maternal Survival

Because the L/M photopigments of Green/Yellow and Red/Yellow trichromats are less spectrally-separated (equivalent to "anomalous" trichromacies in humans) than the photopigments of Green/Red trichromats, it is possible that the former phenotypes might experience lower fitness and bring down the trichromatic group mean. To test for this, we performed the same analyses as those presented above, but this time we limited the sample of trichromats to the Green/Red phenotype.

We found that the results for all of these analyses were qualitatively identical to those presented above for the trichromat-dichromat comparison. There were no significant differences between Green/Red trichromats and dichromats on any of our three measures of female fitness. Specifically, the phenotype of the mother (Green/Red trichromat versus dichromat) has no discernible effect on the length of her IBI ($\chi^2 = 1.41$, df = 1, p = 0.234), and the early death of the first offspring in an IBI leads to a shorter IBI for both Green/Red trichromats and dichromatic mothers ($\chi^2 = 34.0$, df = 1, p<0.001). Second, the mother's vision phenotype does not affect the offspring's survival up to 2 years ($\chi^2 = 2.54$, df = 1, p = 0.109). Third, the survival of females after the age of 2 is not significantly affected by their vision phenotype ($\chi^2 = 3.66$, df = 1, p = 0.055) and the trend is in the direction of dichromats surviving better than the Green/Red trichromats.

Discussion

Although heterozygote superiority appears from the literature to be a widely accepted mechanism explaining primate color vision polymorphism, we found no significant advantage to trichromats over dichromats for the three measures of fitness we examined in female monkeys. Not only did the differences fail to reach significance, the trends for infant and maternal survival were in the opposite direction than predicted by the heterozygote superiority hypothesis (Figures 3 & 4). The one clear and consistent prediction from the literature is that Green/Red trichromats should experience visual advantages (and therefore fitness advantages) in comparison to the five other phenotypes found in capuchins, and in particular Green/Reds should do better than the dichromats [54–60]. However, even when we limited our analyses to the Green/Red trichromat females, there was no indication whatsoever that green/red trichromatic females do better than dichromats on any measure of fitness (fertility rates, offspring survival, maternal survival). We suggest therefore that an alternate selective mechanism is operating to maintain color vision polymorphism in our study animals.

Despite the lack of fitness differences due to color vision phenotype, we did find significant variation in IBIs attributable to the mother's identity, indicating that some aspect of phenotypic quality, other than color vision, is influential in the pace of infant production in our capuchins. These results are in accordance with other recent studies on female primates (e.g., 49]) in which the effect of the mother's identity was investigated. Dominance rank and age are unlikely to explain these characteristic IBI lengths that are consistent over a female's lifetime, in particular because rank and age change over the course of a female capuchin's life and because our previous studies of the effects of dominance and age failed to demonstrate that these variables are significant predictors of IBI length in our study animals [39]. However, other aspects of a female's behavior may affect her life-long fertility pattern, in that some females may consistently behave in ways to enhance their probability and frequency of producing offspring. For example,
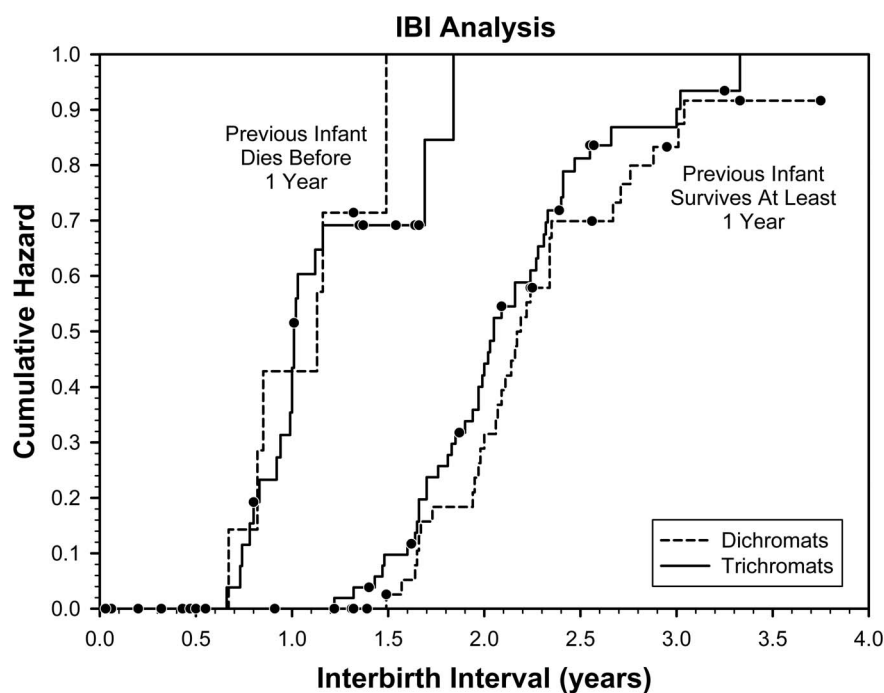
Figure 2. Cumulative Hazard functions for interbirth intervals (IBI) as a function of time (in years) for females with dichromat color vision (dashed lines) and trichromat color vision (solid lines) and for IBIs in which the first infant in the interval did (left lines) or did not (right lines) die at <1 year. Cumulative hazard represents the probability that an inter-birth interval ends on or before a particular age. Dots represent censored IBIs (i.e., those IBIs where the female died prior to the next birth or where at the end of the study period the female had not yet given birth again).
doi:10.1371/journal.pone.0084872.g002
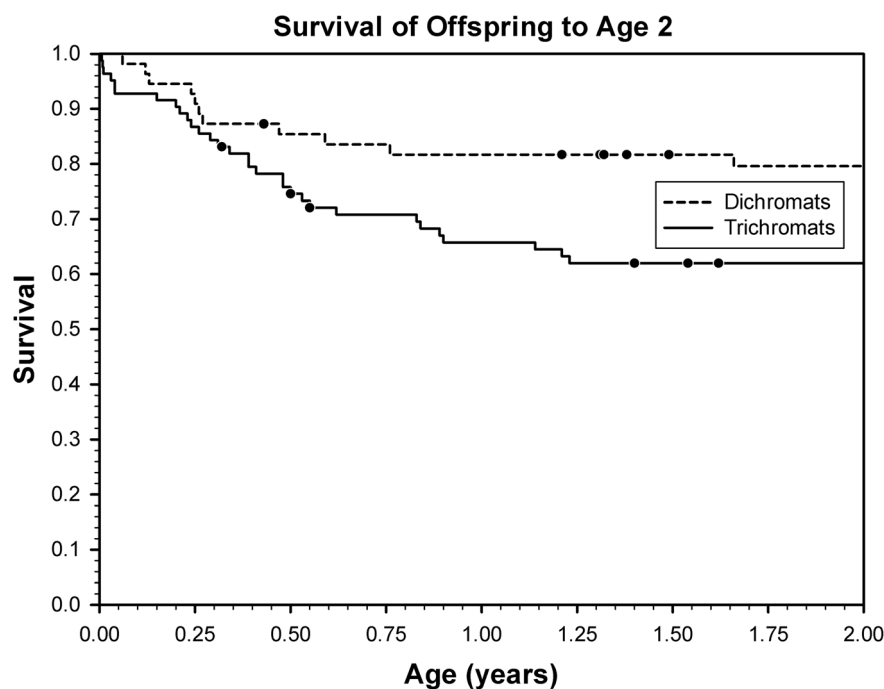
Figure 3. Survival of dependent offspring between birth and age two in years as a function of mother's color vision type (dichromat: dashed line; or trichromat: solid line). Dots represent censored observations (i.e., those individuals that were still alive and less than 2 years of age at the end of the study).
doi:10.1371/journal.pone.0084872.g003
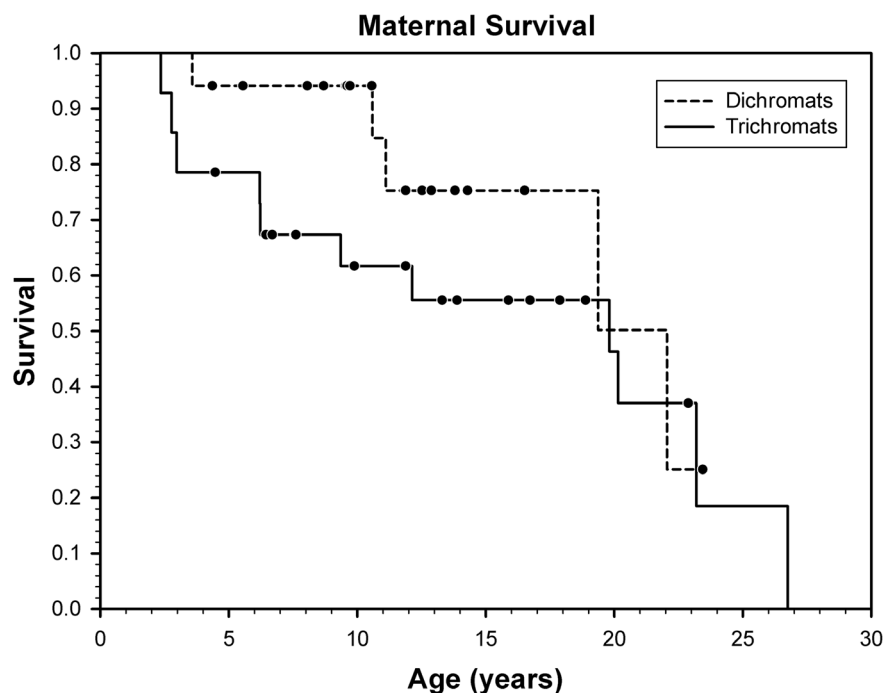
Figure 4. Survival of females from 2 years of age as a function of their color vision type (dichromats: dashed line; trichromats: solid line). Dots represent censored observations (i.e., those individuals that were alive and older than 2 years of age at the end of the study).
doi:10.1371/journal.pone.0084872.g004

they may act strategically to achieve more conceptive matings or to acquire higher-quality resources that enhance fertility.

Thus, this study has demonstrated that there is individual heterogeneity in at least one component of fitness in female capuchins - length of the interbirth interval - but that variability appears not to be driven by a superiority of the trichromatic phenotype, even when we factor out the effects of early infant deaths. Hiwatashi et al. [2] report a clear indication that balancing selection is maintaining color vision polymorphism in these same study animals; yet our analyses show that this polymorphism does not result in differential fitness of trichromats over dichromats, which suggests that evolutionary ecologists should turn their attention to deriving testable predictions from the alternate hypotheses for mechanisms of balancing selection, all of which postulate that multiple color vision phenotypes each have their own advantages under distinct ecological or social conditions.

A surprising dearth of attention has been directed towards evaluating the three alternate mechanisms that have been proposed to explain intraspecific opsin polymorphism: niche-divergence, frequency-dependence, and mutual benefit of association. In part, this may be due to the difficulty of teasing apart these hypotheses due to their largely overlapping predictions. To our knowledge, only one field study has directly addressed an alternate hypothesis. Melin et al. [13] concluded that niche-divergence in diet is unlikely to be operating on polymorphic color vision in Santa Rosa capuchins due to the similarity of diet composition across group members that is driven by cohesive group behaviors. However, the scope of this study was restricted to broad categorizations of food types. A more detailed and nuanced examination of niche differentiation by dichromats and trichromats, including canopy use (e.g., [17,61]) and finer dietary

categorization, may reveal important differences. Furthermore, future work should investigate the extent of communal resource discovery and use, versus individual detection and monopolization of foods. Finally, it will be prudent moving forward to assess the importance of visual tasks unrelated to foraging, such as predator detection, which may be an important factor under a mutual-benefit of association scenario.

In their review article on linking genotypes, phenotypes and fitness, Bradley and Lawler [11] note that it is an open question as to how short-term differences in foraging skills of dichromatic versus trichromatic primates translate to differences in ultimate fitness. Our study suggests that the distinctive foraging skills of dichromatic and trichromatic white-faced capuchins each confer their own advantages such that neither phenotype has an overarching fitness benefit.

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Author Contributions

Conceived and designed the experiments: LMF ADM JFA SK. Performed the experiments: LMF ADM. Analyzed the data: JFA. Wrote the paper: LMF ADM JFA SK.

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