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Colour discrimination learning in black-handed tamarin (*Saguinus midas niger*)

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Abstract Colour is one cue that monkeys use for perceptual segregation of targets and to identify food resources. For fruit-eating primates such as *Saguinus*, an accurate colour perception would be advantageous to help find ripe fruits at distance. The colour vision abilities of black-handed tamarins (*Saguinus midas niger*) were assessed through a discrimination learning paradigm using Munsell colour chips as stimuli. Pairs of chips were chosen from an early experiment with protan and deutan humans. The monkeys (three males and one female) were tested with stimuli of the same hue, but different brightness values, in order to make sure that discriminations were based on colour rather than brightness cues. The results showed that the female, but not the males, presented an above-chance performance for stimuli resembling hue conditions under which tamarins forage (oranges vs greens). Colour vision in *S. m. niger* is discussed according to the advantages and disadvantages of dichromatism in daily search for food as well as to aspects regarding polymorphism in New World monkeys.

Keywords Colour vision · Discrimination learning · Munsell colour chips · *Saguinus midas niger* · Tamarins

Introduction

The minimal requirements for vertebrate colour vision include the presence of more than one spectral mechanism and a means of comparing the outputs from these spectral mechanisms somewhere in the nervous system (Jacobs et al. 1999). Generally, variations in the number of photopigments types found in the retina correlate directly with the dimensionality of colour vision, i.e., two types of cone yield dichromatic colour vision, three types of cone underlie trichromatic colour vision (Jacobs 1996).

The colour vision in different species of mammals has been assessed by microspectrophotometry, electroretinographic flicker photometry (ERG), genetic sequence or through behavioural tasks (review in Jacobs 1993). Apparently, dichromacy is the basic condition for all mammals, with polymorphic trichromacy having probably evolved first in either stem anthropoids or in the ancestor to platyrrhines (Heesy and Ross 2001). This polymorphism, characteristic of many New World monkeys (Jacobs 1998) and present in some prosimians (Tan and Li 1999), allows the existence of dichromatic or trichromatic females but only dichromatic males. In contrast, human beings, apes, Old World monkeys and howler monkeys (genus *Alouatta*) have habitual trichromatic colour vision (Jacobs 1998).

Trichromatic colour vision has long been thought to be the result of an adaptive process involving the detection of targets (i.e., ripe fruits and/or young leaves) against a foliage background (Mollon 1989; Lucas et al. 1998; Sumner and Mollon 2000; Dominy and Lucas 2001). While Dominy and Lucas (2001) claim that leaf consumption has a unique value in maintaining trichromacy in catarrhines, Regan et al. (1998, 2001) have shown that the spectral positioning of the cone pigments found in trichromatic platyrrhine primates is well matched to the task of detecting fruits against a background of leaves.

To our knowledge, only two genera and five species of the family Callitrichidae from a total of five genera, 39 species and 61 subspecies (Rylands et al. 2000) have so far had their colour vision capability investigated (Jacobs et al. 1987; Savage et al. 1987; Tovée et al. 1992; Shyue et al. 1998; Caine and Mundy 2000). The callitrichids form a specialised group of monkeys that fulfils a unique ecological role in the forests of Central and

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South America (Sussman and Kinzey 1984). They feed on three primary types of food items: insects; plant exudates; fruits, flowers and nectar. Tamarins, however, seem to include more fruit in their diet than do marmosets (Sussman and Kinzey 1984).

Data from different species of *Saguinus* indicate that *S. fuscicollis* (Jacobs et al. 1987) and *S. mystax* (Shyue et al. 1998) present a colour vision polymorphism, while *S. oedipus oedipus* (Savage et al. 1987) shows a homogeneous trichromatic behaviour. During the manuscript revision process new evidence about the colour vision of *S. o. oedipus* was published, indicating the existence of a colour vision polymorphism in this species (Jacobs and Deegan 2003). Methodological aspects may account for this contradiction. Truly, *Saguinus* is the most diverse of all platyrrhini genera (Rylands et al. 2000) showing different foraging patterns (Garber 1992). An example of this foraging diversification is found among *S. midas* subspecies. While *S. midas niger* is predominantly frugivorous (87.5% of feeding records) (Oliveira and Ferrari 2000), fruit constitutes only 47% of the diet of *S. m. midas* (Pack et al. 1999). According to Mollon et al. (1984), it is possible that different colour vision phenotypes occupy various locations within the jungle, for example the canopy or relatively open areas at the forest edge. Of all tamarins studied, *S. midas* appears to be the only one to prefer edge to non-edge habitats within the forest (Sussman and Kinzey 1984). Thus, the diversity of feeding behaviours allied to its frugivorous habit make *Saguinus* a genus of great interest for colour vision research.

It has been suggested that a good survey on colour vision perception should include behavioural tests with careful control for brightness cues (Jacobs 1993). Indeed, colour perception is a result of active operations carried out in the nervous system as a whole (Zeki 1999). According to Jacobs et al. (1999), some transgenic mice, diagnosed as being trichromats by ERG and molecular genetics, exhibit a dichromatic behaviour when tested in a visual discrimination task.

Taking this aspect into account and with the aim of contributing to a better understanding of colour vision capabilities in tamarins, we performed a series of experiments in the laboratory using a behavioural paradigm of discrimination learning.

Methods

Subjects

Four adult tamarins (*Saguinus midas niger*), three males and one female, were used in the experiments. The subjects were housed in individual cages (1.0 m width × 1.5 m length × 1.9 m height), maintained and tested in the Primate Centre of the University of Brasilia. Animals were tested in their own home cages in order to avoid the stress inflicted by daily capture and transportation to a novel environment (Savage et al. 1987). They were not food or water deprived. However, during the experimental sessions, food was removed from the cage. The subjects had no previous experience with two-choice colour discrimination training.

Stimuli

The Munsell Book of Colour, containing over 1,600 colour chips, was used to assess colour discrimination abilities. In this system, every colour patch is specified by three attributes of colour: hue, brightness and saturation. Hues are based on ten categories: red (R), yellow-red (YR), yellow (Y), green-yellow (GY), green (G), blue-green (BG), blue (B), purple-blue (PB), purple (P), and red-purple (RP). Each hue category has four different spectral points: 2.5, 5, 7.5, and 10. The hue is represented by a number and a letter, whereas a fraction stands for brightness over saturation (e.g. the notation 7.5R 6/10 corresponds to a 7.5 red colour chip, with brightness 6 and saturation 10). The Munsell system is a suitable method for testing colour discrimination in endangered species (Savage et al. 1987; Gomes et al. 2002).

To select the pairs of Munsell colour chips and to estimate the degree of difficulty of each pair, human subjects were used in two-choice colour discrimination tests, using the same stimuli and apparatus previously employed with monkeys (see Gomes et al. 2002).

To make sure that discriminations were based on colour rather than brightness cues, the animals were tested with stimuli of the same hue but different brightness values (Savage et al. 1987). Each pair of stimuli was accomplished by eight colour chips: four different brightness levels from a determined hue paired against four different brightness of another hue. This resulted in a total of 16 different possible combinations.

Apparatus

A plexiglas version of the Wisconsin General Test Apparatus (Harlow and Bromer 1938) was mounted in front of the subject's home cage. This apparatus consisted of a portable tray, two stimuli-holders and a movable screen. The portable tray had two food wells, spaced 12 cm apart, designed to hold the rewards (pieces of grapes or raisins). Stimuli-holders (3 × 5 × 2 cm) could be positioned to hide the food wells. These stimuli-holders had a 1.2-cm diameter round window in their upper surface, allowing visualisation of stimuli by the subjects. The movable screen prevented the monkeys from observing the stimuli between trials.

Procedure

The experimental sessions were conducted 3 times a week, from 10:00 am to 12:00 pm, under natural daylight diffuse illumination.

Training phase began after the subjects had learned how to manipulate the stimuli-holders and pick-up the reward. The animals had to perform a two-choice discrimination task between a positive discriminative stimulus (SD+, the rewarded stimulus) and a negative discriminative stimulus (SD−, never rewarded). In this phase, only pairs of stimuli of easy discrimination by protans and deutans were used. The subjects were rewarded when they displaced the stimuli-holder containing the SD+. A non-correction procedure was used; i.e., following an incorrect choice the screen was lowered, and another trial begun. The intertrial interval was about 15 s. A variable number of trials was made on each training session, having a total duration of 40 min. The left or right position of the SD+ was determined according to the Gellerman table of random numbers (Gellerman 1933).

After reaching the criterion of 80% correct responses, the animals proceeded to the testing phase. In this phase, subjects were faced with a two-choice discrimination task involving the same SD+ of the training phase and five different SD−. Some of these pairs of stimuli were: (1) of easy discrimination by trichromats and dichromats ("easy pairs"); (2) of easy discrimination by trichromats and difficult discrimination by dichromats ("difficult pairs"); or (3) of difficult discrimination by trichromats and dichromats ("impossible pairs"). The "easy pairs", were out of the dichromats confusion range and represented the pairing of purples, blues and

reds (7.5P, 5PB, 2.5PB, 5B, 7.5B, 10BG, 5R) with oranges (2.5YR, 7.5YR, 10YR). The "difficult pairs" were composed by greens (7.5GY; 2.5G; 5GY) versus oranges (2.5YR; 7.5YR; 10YR). The "impossible pair" was constituted by pairing oranges (10YR n/10) against oranges (10YR n/8), making their discrimination unattainable. As stated before, each brightness level of the SD+ was paired to each brightness level of the SD−, adding up to 16 pairs. These pairs were presented 3 times each, totalling 48 trials per experimental session. Based on these 48 trials, a percentage of correct responses was determined. After finishing the testing phase, the subjects were introduced into another training phase, with a different SD+.

Statistical analyses

The binomial test was used to construct the 95% confidence limits around chance performance based on the number of test trials. For 48 test trials, the upper limit was determined as 65%. The performance of all subjects was compared to these confidence limits, and any performance above the upper limit was significant ($P < 0.025$).

Colour measurement

To evaluate the colour difference between the SD+ and SD− used in the experiments an Ocean Optics USB2000 spectrophotometer was used. The equipment was calibrated against an absolute black and then against a standard white ($BaSO_4$). Spectral reflectance was determined every 0.36 nm. All measurements were made directly from the colour chips, at a distance of 10 mm, with usage of a Xenonium light source (PX2, Ocean Optics). A free version of the Munsell Conversion Software (GretagMacbeth) was used to convert Munsell notation to CIELab values (L^*, a^*, b^*). Total spectral differences ($\Delta E^*ab = ((\Delta L^*)^2 + (\Delta a^*)^2 + (\Delta b^*)^2)^{1/2}$) were determined following Caine and Mundy (2000).

Results

Colour data

The reflectance spectra of the colour chips are shown in Fig. 1. As expected, large differences were found between "easy pairs". 7.5YR (orange): CIE L^* 56.5, a^* 17.4, b^* 45.4; 5B (blue): CIE L^* 56.5, a^* −17.3, b^* −19.2; total difference = $73.3\Delta E^*ab$. Similarly, large differences were found for the orange chips and the green chips ("difficult pairs"). 2.5YR (orange): CIE L^* 56.5, a^* 34.3, b^* 47.3; 7.5GY (green): CIE L^* 56.5, a^* −31.3, b^* 43.2; total difference = $65.7\Delta E^*ab$. 7.5YR (orange): CIE L^* 56.5, a^* 17.4, b^* 45.4; 2.5G (green): CIE L^* 48.8, a^* −50.7, b^* 26.7; total difference = $71.0\Delta E^*ab$. 10YR (orange): CIE L^* 66.6, a^* 12.2, b^* 63.8; 5GY (green): CIE L^* 66.6, a^* −29.0, b^* 61.6; total difference = $43.1\Delta E^*ab$. There were smaller differences between the "impossible pair" stimuli. 10YR n/10 (orange): CIE L^* 66.6, a^* 12.2, b^* 63.8; 10YR n/8 (orange): CIE L^* 66.6, a^* 10.0, b^* 51.3; total difference = $12.7\Delta E^*ab$.

Behavioural experiments

The results show a difference in performance between male and female subjects. Figures 2, 3 and 4 show the results when the SD+ was 2.5YR, 7.5YR and 10YR, respectively. In each case, all four subjects presented a performance above chance (65%) for the "easy pairs" (first three pairs and the fifth pair; see Figs. 2, 3, 4). However, in the "difficult pair" (fourth pair), male subjects showed a performance below chance whereas the female subject performed significantly above chance level.

Figure 5 illustrates the tamarins' performance when 10YR was used as the SD+. The subjects performed above chance in the "easy pairs". However, for the "impossible pair" (fourth pair) all subjects presented a performance below chance.

Discussion

The results obtained in the present study demonstrate that all subjects presented a performance above chance in the "easy pairs" (baseline pairs), indicating an efficient learning in the two-choice discrimination task. For the "difficult pairs" (colour vision diagnostic pairs), a difference in performance was noticed between subjects. Whereas the female subject accomplished the discrimination of all "difficult pairs", the male tamarins showed a chance level performance. However, in the "impossible pair" (control pair), the performance of all tamarins was significantly below chance, indicating that the subjects were not able to make any discrimination in the absence of hue cues.

The different performances presented by the subjects in the "difficult pairs" indicate the presence of distinct kinds of colour perception in *S. m. niger*. A parallel between the tamarin's perception and human perception can be traced. The visual perception presented by the female tamarin matches closely the performance of human trichromats (Gomes et al. 2002), that discriminated all but the "impossible pair". Moreover, the three male tamarins and human dichromats showed the same points of spectral confusion (Gomes et al. 2002), being unable to discriminate the "difficult pairs".

In the present study, we managed to present an ecologically relevant task to the animals. Indeed, yellow to orange fruits seem to predominate in the diet of many neotropical primates. Terborgh (1983) found that 62% of fruit eaten by New World monkeys belonged to the yellow / orange / red region of the spectrum. Similarly, 54% of the fruit consumed by *Saguinus* are in the yellow / orange / red range (Savage et al. 1987). Therefore, the hues used as SD+ and SD−, in the "difficult pairs", resembled the hues of the mostly consumed fruits by *Saguinus* and the hues of the foliage background. A recent study has also used, with success, stimuli in this spectral range to assess the colour vision in dichromatic and trichromatic marmosets (Caine and Mundy 2000).

Concerning the ability to spot targets (fruits and leaves) against a foliage background, trichromacy offers a significant advantage over dichromacy (Regan et al. 1998; Caine and Mundy 2000; Sumner and Mollon 2000; Dominy and Lucas 2001). Since fruits comprise

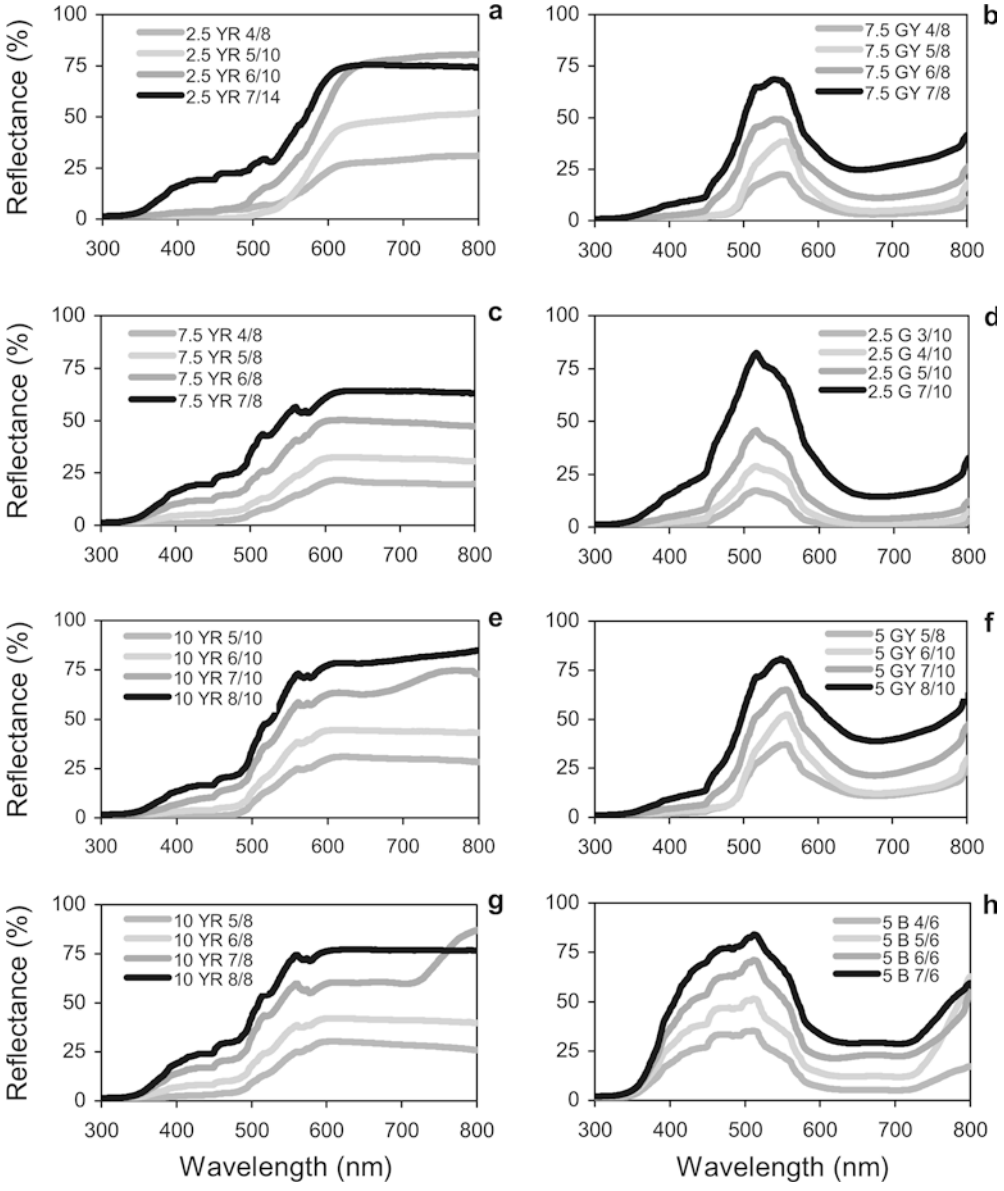

Fig. 1a–h Reflectance spectra of Munsell colour chips. Each panel shows data for four colour chips of the same hue and different brightness/saturation. **a** 2.5YR (*orange*); **b** 7.5GY (*green*); **c** 7.5YR (*orange*); **d** 2.5G (*green*); **e** 10YR n/10 (*orange*); **f** 5GY (*green*); **g** 10YR n/8 (*orange*); and **h** 5B (*blue*)

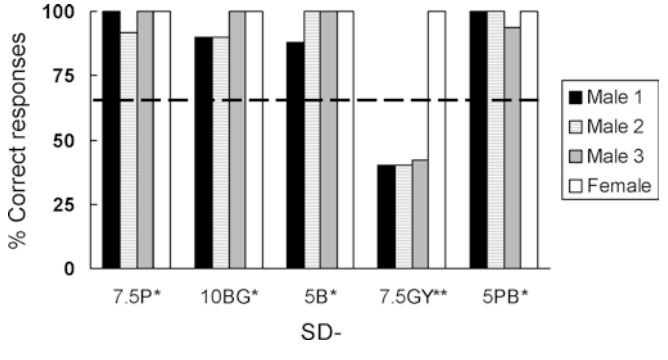

Fig. 2 Colour discrimination performance in *Saguinus midas niger*, with the Munsell chip 2.5YR (*yellow-red*) as positive discriminating stimulus (SD+). The negative discriminating stimuli (SD−) are disposed along the *abscissa*: 7.5P (*purple*), 10BG (*blue-green*), 5B (*blue*), 7.5GY (*green-yellow*), 5PB (*purple-blue*). The fourth arrangement (2.5YR vs 7.5GY) constitutes the "difficult pair". The *horizontal line* indicates the upper limit (65% of correct responses) of 95% confidence interval for discriminative performance. * easy pair; ** difficult pair

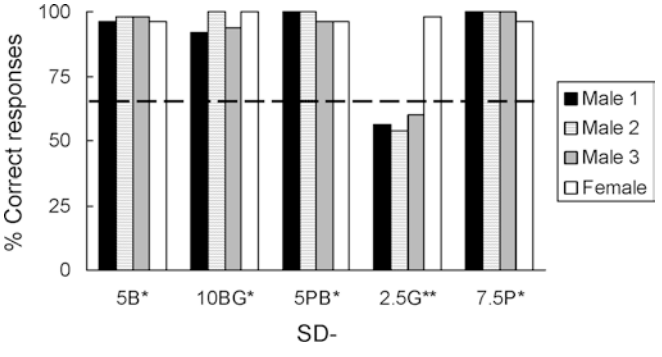

Fig. 3 Colour discrimination performance in *Saguinus midas niger*, with the Munsell chip 7.5YR (*yellow-red*) as positive discriminating stimulus (SD+). The negative discriminating stimuli (SD−) are disposed along the *abscissa*: 5B (*blue*), 10BG (*blue-green*), 5PB (*purple-blue*), 2.5G (*green*), 7.5P (*purple*). The fourth arrangement (7.5YR vs 2.5G) constitutes the "difficult pair". Other conventions as in Fig. 2

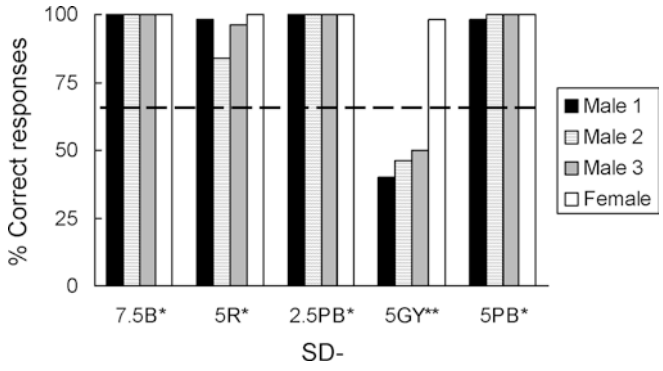

Fig. 4 Colour discrimination performance in *Saguinus midas niger*, with the Munsell chip 10YR (*yellow-red*) as positive discriminating stimulus (SD+). The negative discriminating stimuli (SD−) are disposed along the *abscissa*: 7.5B (*blue*), 5R (*red*), 2.5PB (*purple-blue*), 5GY (*green-yellow*), 5PB (*purple-blue*). The fourth arrangement (10YR vs 5GY) constitutes the "difficult pair". Other conventions as in Fig. 2

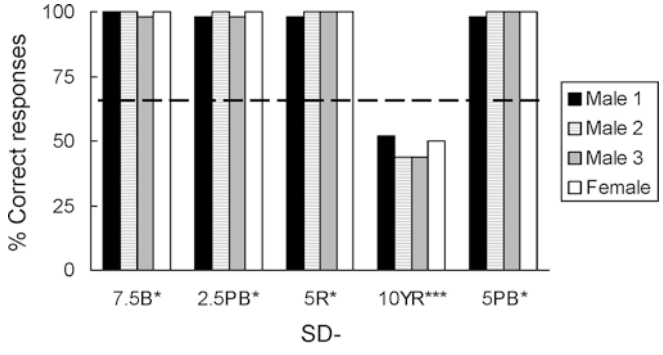

Fig. 5 Colour discrimination performance in *Saguinus midas niger*, with the Munsell chip 10YR (*yellow-red*) as positive discriminating stimulus (SD+). The negative discriminating stimuli (SD−) are disposed along the *abscissa*: 7.5B (*blue*), 2.5PB (*purple-blue*), 5R (*red*), 10YR (*yellow-red*), 5PB (*purple-blue*). The fourth arrangement (10YR vs 10YR) constitutes the "impossible pair". * easy pair; *** impossible pair. Other conventions as in Fig. 2

the major portion of diet in many New World monkeys (Savage et al. 1987), including the genus *Saguinus* (Egler 1993), what would maintain the remarkable polymorphism of cone pigments that is found in platyrrhine monkeys?

As mentioned by Caine and Mundy (2000), the advantages of trichromacy in locating food in the red / orange part of the spectrum may not generalise to all conditions under which primates forage. Comparing the visual environment of an Old World forest in Uganda (Sumner and Mollon 2000) with that of a New World forest in French Guiana (Regan et al. 2001), it was found that fruits have different characteristics. While in Uganda fruits are small and darken when they ripen, in New World forest they are larger and lighten during ripening. Thus, the platyrrhine diet would be easier to segregate from the foliage background in comparison to the catarrhine diet, reducing the selective advantage of trichromacy over dichromacy in South America (Sumner and Mollon 2000).

The results of the present study favour the hypotheses of the obligatory dichromatism in males (Mollon et al. 1984) as it happens for other species of callitrichids (Jacobs et al. 1987; Tovée et al. 1992; Shyue et al. 1998; Caine and Mundy 2000). However, additional experiments are necessary to enlarge the sample size and to determine the genetic basis of colour vision in *S. m. niger*. Moreover, since there are around 15 species of *Saguinus* and more than 200 species and subspecies of neotropical primates (Rylands et al. 2000), further work is necessary in order to determine if colour vision polymorphism is a general feature of platyrrhine species.

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