Primates (2003) 44: 171–176
DOI 10.1007/s10329-002-0017-5

ORIGINAL ARTICLE

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Behavioral evidence of color vision deficiency in a protanomalia chimpanzee (*Pan troglodytes*)

Received: 17 September 2002 / Accepted: 21 November 2002 / Published online: 27 February 2003

Abstract Although color vision deficiency is very rare among Old World monkeys and apes, one male chimpanzee (Lucky) was identified as protanomalous by genetic and physiological analyses. This study assessed behavioral phenotypes of Lucky and four chimpanzees with normal color vision by discrimination task using the modified Ishihara pseudo-isochromatic plates. Lucky could not discriminate the stimuli that the other chimpanzees could. This is the first behavioral evidence of color vision deficiency in chimpanzees.

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Keywords Color vision · Chimpanzee · *Pan troglodytes* · Color vision deficiency

Introduction

Most mammals have dichromatic color vision. Among primates, many New World monkeys exhibit a polymorphism of color vision: some animals are dichromatic, others trichromatic. On the other hand, humans, apes, Old World monkeys and one New World monkey (howler monkeys) have habitual trichromatic color vision. Their trichromatic color vision originates from three types of retinal cone photoreceptors possessing different spectral sensitivities. This difference arises from the selective expression of three genes encoding long-wavelength-sensitive (L), middle-wavelength-sensitive (M), and short-wavelength-sensitive (S) opsins.

In humans, although the gene encoding S opsin is located on chromosome 7, the genes encoding L and M opsins are located in a head-to-tail tandem array on the X chromosome. In addition, the M and L opsin genes have high nucleotide sequence similarity (Nathans et al. 1986; Yokoyama and Yokoyama 1989). These two structural features promote frequent unequal meiotic recombinations, thus altering the gene array and the subsequent identity and nature of the M and L opsins. In fact, inherited red/green color vision deficiencies are frequent in humans, and these deficiencies are due to variations in the structure and combinations of M and L opsin genes. The incidence of such color vision deficiencies reaches up to 7–8% of males in some human populations (Kalmus 1965; Pokorny et al. 1979). Although the underlying mechanisms and nature of color vision seem very consistent among Old World monkeys and apes, the frequency of such variation is quite low (Jacobs and Williams 2001; Onishi et al. 1999, 2002; Terao et al., in preparation).

To date, only a few cases of color vision deficiency in the Old World monkeys and apes have been identified using molecular biological techniques to specify the genotype. Three male crab-eating macaques (*Macaca fascicularis*) were found in the Pangandaran National Park of Java Island, Indonesia. They have the same genotype of a single hybrid gene, *R4G5*, on the X chromosome and they are thought to be protans because the absorbance spectrum of *R4G5* photopigment is very close to that of the M photopigment (Onishi et al. 1999, 2002). One male chimpanzee (*Pan troglodytes*) was found in a colony of Sanwa Kagaku Kenkyusho Kumamoto Primate Park, Japan. He has a hybrid gene, *R4G5*, and a normal M opsin gene, thus is considered to have protanomalous trichromatic color vision (Terao et al., in preparation). Their physiological phenotypes determined by investigating their relative spectral sensitivity were confirmed to agree with the result of the genetic analysis (Hanazawa et al. 2001; Mikami et al. 2003; Terao et al., in preparation).

The aim of the present study was to confirm the behavioral evidence of color vision deficiency in chimpanzees with a hybrid gene. To achieve the above purpose, a discrimination task was conducted. Stimuli were prepared by modifying the Ishihara pseudo-isochromatic plates. The Ishihara pseudo-isochromatic plates were devised for identifying color vision deficiency in humans. In a plate, the target and background are formed from discrete patches, and the lightness of individual patches is varied rather than being equated. These features are used to avoid the difficulty of generating equiluminant edges. It has been verified that the Ishihara test is an effective method to distinguish between normal and deficient red-green color perception in humans (Birch 1997).

Methods

Subjects

Five male chimpanzees kept at the Sanwa Kagaku Kenkyusho Kumamoto Primate Park, Japan, were used as subjects in this study. Their genotypes were determined by Terao et al. (in preparation). One individual (Lucky, 28 years old) had one hybrid gene, *R4G5*, which consisted of the 5′ part of the L opsin gene and the 3′ part of the M opsin gene at the first position, and one normal M opsin gene at the second position of the gene array. Another individual (Aruku, 12 years old) had one L opsin gene at the first position, one M opsin gene at the second position, and one hybrid gene, *G4R5*, which consisted of the 5′ part of the M opsin gene and the 3′ part of the L opsin gene at the third position of the gene array. Since it is known that in humans, only the first two genes of the tandem array are effective, this hybrid gene located in the third position may not influence Arukus's color vision. The other individuals, Jiro (13 years old), Mizuo (12 years old), and Kotetsu (12 years old) had one L opsin gene and one M opsin gene; therefore, they were considered to have normal color vision. Lucky, Aruku, and Jiro were examined for their retinal chromatic sensitivity, and it was shown that Lucky exhibited severely diminished retinal sensitivity to long-wavelength lights, as compared with the other normal chimpanzees (Mikami et al. 2003).

All subjects lived in outdoor enclosures and indoor cages connected to each other. Their housing conditions and other information are summarized in Table 1.

Stimuli and apparatus

Five stimulus sets, P100, E50, E25, E12, and E0, were prepared (Fig. 1). Each set contained two stimuli. One (S+) contained a ring consisting of red-brown dots. The other (S−) did not contain it. The diameter of each stimulus was 9 cm. The inner and outer diameters of the ring were 5 cm and 6.5 cm respectively. Each stimulus was printed out on glossy paper (Kokuyo, KJ-AG1315) using an ink-jet printer (Epson, PM-800C).

The S+ of P100 contained a dark red ring dappled against light green. This stimulus could be discriminated from S− of P100 by both normal trichromats and individuals with color vision deficiency. The ring in the S+ of E0 consisted of red-brown dots that varied in lightness in two levels dappled against green dots that varied in lightness in three levels. These were confusing color for human protanopes, and the mosaic arrangement prevented them from using lightness as a cue. Accordingly, it was supposed that an individual who had protanopic color vision could not discriminate S+ from S− of E0. The stimulus sets E50, E25, and E12 were prepared by dither mixing of P100 and E0. The ratios of P100 in E50, E25 and E12 were 50%, 25%, and 12%, respectively. Images were processed using Adobe Photoshop 5.5. The colors in the stimulus E0 and P100 were measured under a fluorescent light for color measurement (Toshiba Light & Technology, FL20S.D-Edl-D65) with a Minolta Chroma Meter CS-100. The results are shown in Fig. 2. Each stimulus was placed in a transparent soft card case attached to the bottom of a turned down opaque plastic plate (11 cm in diameter, 5 cm in depth).

A modified version of the Wisconsin General Test Apparatus (WGTA) was used. The apparatus was a horizontal tray (about 60×30 cm) mounted on a basket (51.5×36.0×30.5 cm) or a box (59.0×39.0×11.0 cm). The basket was used for Lucky, Aruku and Jiro, and the box was used for Mizuo and Kotetsu according to the structures of their cages. The tray containing the stimuli was placed in front of the experimental cage. Two plates were placed upside down on the tray 2 cm apart from each other, and plastic cases containing stimuli were mounted on the bottoms of the plates. A rectangular opaque box (57.0 cm wide x 17.0 cm high x 27.0 cm deep with a top, a front, and two side panels) was used to allow the experimenter to set up problems and prevent subjects from viewing the stimuli between trials. The face of the experimenter was covered with a face shield (face mask), so that the subject could not use the eye direction or facial expression of the

Table 1 Subjects' genotype, age, origin, and housing conditions

Subject	No.	Genotype	Sex	Age	Birthday	Origin and subspecies	Indoor	Outdoor
Lucky	948	5′-R4G5-M-3′	Male	28	1974 ?	Wild (West Africa, *verus* ♂)	With one male	
Aruku	1,098	5′-L-M-G4R5–3′	Male	12	7/12/1989	Captive (*verus* ♂× *verus* ♀)	Alone	With one male
Jiro	1,083	5′-L-M-3′	Male	13	11/11/1988	Captive (? ♂× *verus* ♀)	With two males and two females	
Mizuo	1,095	5′-L-M-3′	Male	12	2/11/1989	Captive (*verus* ♂× *verus* ♀)	Alone	With one female
Kotetsu	1,132	5′-L-M-3′	Male	12	31/1/1990	Captive (*verus* ♂× *schweinfurthii* ♀)	Alone	With two male and two females

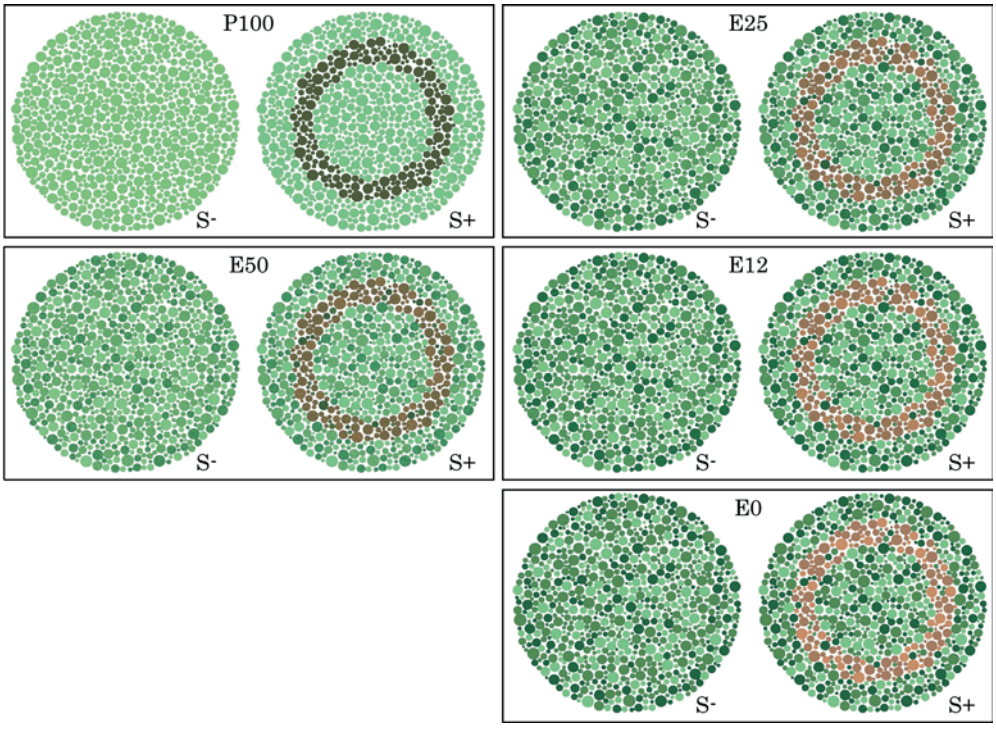

Fig. 1 Stimulus sets for experiment prepared by modifying the Ishihara pseudo-isochromatic plates. These stimuli were used to identify dichromatic macaques

experimenter as a cue to find the correct side. The same fluorescent light used for color measurement (Toshiba Light & Technology, FL20S.D-Edl-D65) was placed 75 cm from the floor above the stimuli.

Procedure

The experiments were conducted individually in indoor cages. One or two sessions were conducted every day for each subject between 1030 and 1600 hours.

One session consisted of 20 trials. Trials started with the presentation of one stimulus set at a time. On any given trial, the subject was required to discriminate between two stimuli. The reward (a piece of fruit, such as apples, bananas, oranges, and pineapples) was placed under S+, and the experimenter gave the reward only after the subject touched the plate carrying S+. The positions of the reinforcement were altered pseudo-randomly. Inter-trial intervals were about 30 s.

The percentages of correct responses were calculated for each session in the training phases and in the control phase, and for each stimulus set in the test phase.

Training phase

In the first training phase, the stimulus set P100 was used. This training continued until the percentages of correct responses were more than 90% in three successive sessions.

In the second training phase, the stimulus set E50 was used. This training continued until the same criterion as the first training phase was reached.

Test phase

Ten test sessions were conducted for each individual. Each test session consisted of 17 baseline trials and three probe trials. In the baseline trials, the stimulus set E50 was presented. In the three probe trials, E25, E12 and E0 were presented. The reward was placed under S+, and the experimenter gave the reward after the subject touched the plate carrying S+ as in the training phases. The probe trials were mixed with baseline trials pseudo-randomly.

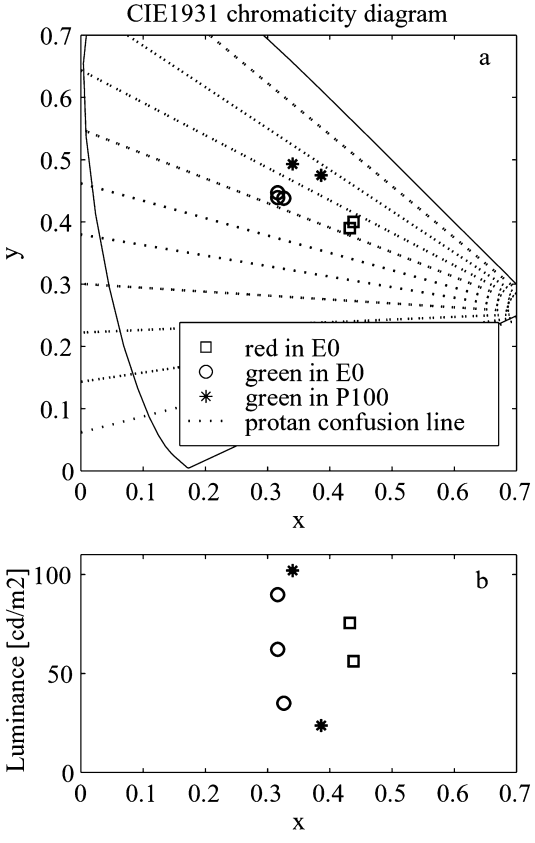

Fig. 2a, b Chromaticity and luminance of color elements in E0 and P100. **a** The *horizontal* and *vertical axes* show *x* and *y* values of CIE1931 chromaticity diagram, respectively. Both reds (*squares*) and greens (*circles*) are located along the same protan confusion line. **b** The *vertical axis* shows the luminance and the *horizontal axis* is the same as in **a**

Control phase

One control session was conducted for each individual after the test sessions. In the control session, the back of the stimulus set E50 was presented and the reward was placed under S+. The purpose of this phase was to confirm that subjects solved the task not by seeing the action of the experimenter or by smelling pieces of fruit but by comparing the stimuli, since under this condition, both sides of stimuli were plain white sheets and the subjects could not see S+.

Analysis

The performance of each subject in probe trials was analyzed by one-tailed binomial test.

Results

Training phase

Figure 3 shows the percentage of correct choices by the subjects in the first and second training phases. In the first training phase, Aruku, Mizuo and Kotetsu fulfilled the criterion in 20 sessions. On the other hand, Lucky and Jiro took more than 40 sessions before doing so. This might be caused by their lack of attention since their indoor cages were placed where they could see female chimpanzees, and they were not used to being alone. In the second training phase, although four subjects fulfilled the criterion quickly, Lucky took more than 30 sessions. As shown in the graph, his percentage of correct choices was more than 70% in most sessions. Thus, although Lucky could not reach our target, we believe that he could understand the task and he simply could not discriminate S+ from S− because of his lack of attention.

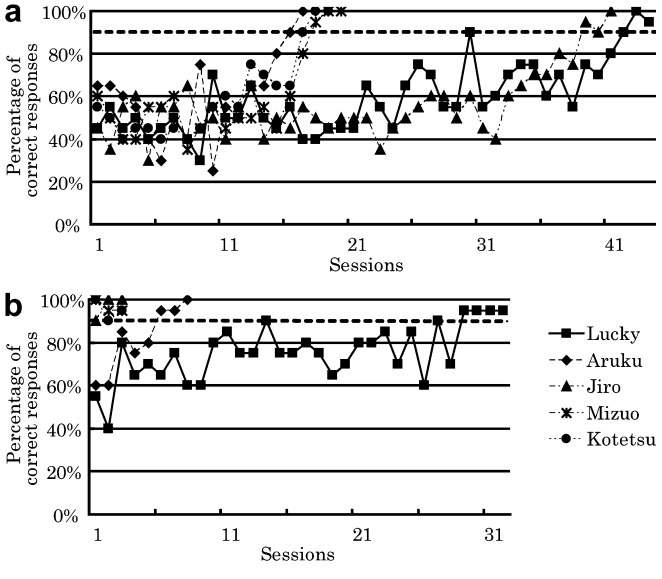

Fig. 3a, b Percentage of correct responses in the fist training phase (**a**) and the second training phase (**b**). The *vertical axis* shows the percentage of correct responses in each session and the *horizontal axis* shows the order of sessions in each training phase

Baseline trials and control session

Figure 4 shows the percentage of correct choices by the subjects in baseline trials of the test phase and in the control session. All subjects showed more than 80% correct choices in baseline trials during the ten test sessions. Thus, all subjects could solve the task during the test sessions regardless of their genotypes. On the other hand, in the control sessions, none of the subjects' choices were significantly different from chance. This result means that all subjects did not use the action of the experimenter or smell of fruits to solve the task.

Probe trials

Figure 5 shows the number of probe trials in which each subject chose correctly. Under all conditions (E25, E12 and E0), Aruku, Jiro, Mizuo and Kotetsu had percentages of correct choices that were significantly different

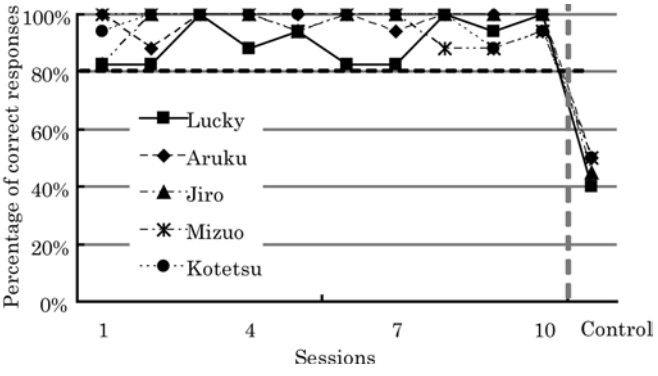

Fig. 4 Percentage of correct responses of baseline trials in each test session and in the control session. The *vertical axis* shows the percentage of the correct responses of baseline trials in each session and the *horizontal axis* shows the order of sessions in the test phase and the control session

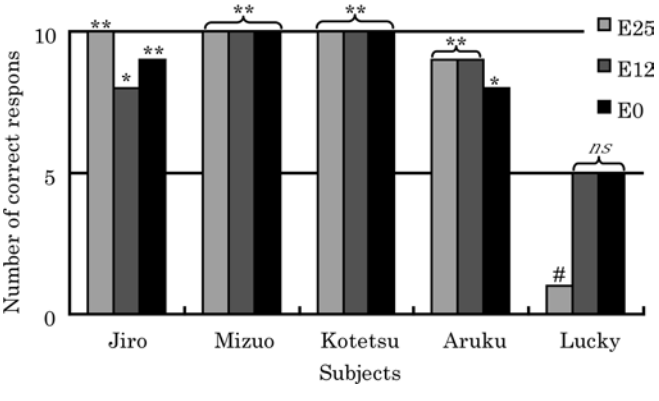

Fig. 5 Number of correct responses to each stimulus set in probe trials. The *vertical axis* shows the number of correct responses and the *horizontal axis* shows the subjects. The *asterisks* indicate that the subject's number of correct responses was significantly high (*$P = 0.0547$; **$P < 0.05$). The *sharp mark* (#) indicates that the subject's number of wrong responses was significantly high ($P < 0.05$)

from chance ($P = 0.0547$ for E12 of Jiro and E0 of Aruku, $P < 0.05$ for others). By contrast, Lucky's percentage of correct choices was not significantly different from chance under the conditions of E12 and E0. Under the conditions of E25, however, Lucky's percentage of wrong choices was significantly high ($P < 0.05$).

Discussion

In this experiment, the behavioral difference between Lucky and the other chimpanzees was clearly demonstrated in the discrimination task using WGTA. Lucky could not make the correct choice in probe trials in which other chimpanzees could. Lucky's percentage of wrong choices was significantly high under the E25 condition. This means that Lucky might be able to discriminate E25 using some partial cues. However, since he selected S− instead of S+, he could not regard S+ of E25 as being the same as S+ of E50. In addition, Lucky could not discriminate S+ from S− under E12 and E0 conditions at all. Thus, Lucky could not detect the ring composed of colors that were confusing to human protanopes. These behavioral results of our experiment clearly show that Lucky was color vision deficient. Aruku, who had one hybrid gene (*G4R5*) at the third position of the gene array, could make the correct choice in probe trials like other chimpanzees with normal color vision. From these results, the data that only the first two genes of the tandem array are effective was confirmed not only in humans but also in chimpanzees. That is, the results of the genetic analyses are consistent with the behavioral phenotypes.

It is commonly assumed that a primary advantage of trichromacy is the ability to locate colored fruits or young leaves when the dappled illumination and variegated background of the forest make it difficult to use form or lightness cues. Some studies suggested that the spectral positions of three Old World primate pigments are optimal for finding fruits or young leaves against a background of mature leaves (Dominy and Lucas 2001; Lucas et al. 1998; Osorio and Vorobyev 1996; Regan et al. 1996, 1998; Sumner and Mollon 2000). However, before proceeding to the argument on the advantages of trichromacy, we need to confirm the following assumption. The difference of opsin genes will cause the difference in color vision. This individual difference in color vision will affect individual behavior. Since selective pressure affects the behavior of individuals, it is necessary to confirm whether behavioral phenotypes agree with the results of genetic analyses and to compare the behavior between individuals with color vision deficiency and those with normal trichromacy. We could provide basic data on this topic. In New World monkeys that exhibit a polymorphism of color vision, the superiority of trichromats in discrimination ability from middle to long wavelength has been shown by laboratory psychophysical tests (squirrel monkeys: Jacobs 1984; Mollon et al. 1984; tamarins: Jacobs et al. 1987; marmosets: Tovee et al. 1992). Moreover, one study attempted to demonstrate the superiority of trichromats by comparing the foraging ability of dichromatic and trichromatic animals (marmosets) (Caine and Mundy 2000). For macaques and chimpanzees, it is necessary to investigate the foraging behavior of individuals with normal color vision and color vision deficiency and to investigate how individuals with the color vision deficiency adapt to their natural environment and how this process differs from that in individuals with normal color vision.

Furthermore, factors that prompted the evolution of trichromatic color vision can also be investigated by another approach: comparing ecological conditions between species whose incidences of color vision deficiency are different may be useful and may lead to understanding the factors that affect the evolution of trichromatic color vision. To date, the incidence of the color vision deficiency in chimpanzees calculated from our data is 1/62(= 1.6%). We have identified several individuals who carry the hybrid gene of M/L color photopigment. They are thought to originate from different families in the wild (Table 1, Terao et al., in preparation). In addition, there are two types of the hybrid gene. Considering these findings, chimpanzees with the color vision deficiency could exist in the wild, and the frequency of chimpanzees with color vision deficiency is probably lower than that of humans and higher than that of macaques. If this were true, we could identify the factors and advantages of trichromatic color vision by comparing their ecology. To this end, it is necessary to survey the more accurate frequency of color vision deficiency in chimpanzees and also to survey the frequencies of color vision deficiency in other primate species.

Acknowledgements This study was supported by the cooperation research program of Primate Research Institute, Kyoto University, Japan Society for the Promotion of Science (no. 14–08373) and Grant-in-Aid for Specially Promoted Research (no. 10CE2005). All experimental procedures were approved by the Animal Committee of the Sanwa Kagaku Kenkyusho Kumamoto Primate Park and were in accordance with Guide for the Care and Use of Laboratory Primates (1986, 1996, 2001) of the Primate Research Institute of Kyoto University and Guidelines for Care and Use of Laboratory Animals (1985) of the National Institute of Health.

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