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ORIGINAL PAPER

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Learning and discrimination of colored papers in jumping spiders (Araneae, Salticidae)

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Abstract Color discrimination in jumping spiders *Hasarius adansoni* was examined by heat-avoidance learning in association with colored papers. The arena for the experiment was divided into two halves by a pair of colored papers. The colored papers used in this study were blue, green, yellow, red, white, gray and black. In training sessions, one half of the arena was heated from the bottom by a hot plate, and freely walking spiders were individually trained to avoid the heated half. In subsequent memory tests without heat, they consistently avoided the heat-associated colored papers. We found that jumping spiders could learn blue-green, blue-yellow, blue-red, blue-gray, green-yellow, green-red, green-gray, yellow-red, yellow-gray and red-gray patterns. Moreover, spiders trained with a blue-white pattern, a green-white pattern, a yellow-white pattern or a red-white pattern could discriminate the blue, green, yellow or red from black. It seems that jumping spiders can discriminate the blue, green, yellow and red papers by their hue, although brightness may also be used together with the color cue to discriminate colored papers.

Key words Heat-avoidance learning · Color vision · Jumping spider

Abbreviations *AL* anterior lateral · *AM* anterior median · *B* blue · *Bk* black · *C* control · *CR* choice rate · *G* green · *Gy* gray · *PL* posterior lateral · *PM* posterior median · *R* red · *T* test · *W* white · *Y* yellow

Introduction

Jumping spiders, which are diurnal hunting species, have four pairs of eyes on the prosoma. These eyes are referred to as the anterior median (AM), anterior lateral (AL), posterior median (PM) and posterior lateral (PL) eyes (for reviews see Blest 1985; Forster 1985; Land 1985; Yamashita 1985). A number of behavioral activities of the jumping spiders are initiated by visual stimuli. The AM eyes play a significant role in the initiation of behavioral events and are named the principal eyes. Homann (1928) and Crane (1949) showed that blinding the AM eyes results in a cessation of prey-catching as well as courtship behavior, while blinding the AL and PL eyes affects only the initial turn toward an object moving outside the visual field of the AM eyes.

Jumping spiders do possess some specific colorful adornments. Peckham and Peckham (1894) showed that painting various parts of live female jumping spiders in atypical colors greatly reduces male sexual display. Crane (1949) found that the yellow patches on the clypeus of *Corythalia xanthopa* are essential for the release of threat display. A crude two-dimensional model with similar yellow patches initiated threat display, while a model with white patches did not. Kästner (1950) observed that *Evarcha falcata* leaps upon and climbs up a striped wall. Black and white stripes elicited the behavior most effectively, while black and gray stripes were only weakly effective. On the other hand, colored (blue or orange) and gray stripes were equally as effective as black and white stripes. Kästner (1950) concluded that the AM eyes are capable of color discrimination. Land (1969) observed that the retina of the AM eyes of the jumping spiders, *Phidippus johnsoni* and *Metaphidippus aeneolus*, consists of four layers of receptors (1, 2, 3, and 4 from the deepest layer forward), and showed that red light from infinity focused on layer 1, blue-green on 2, violet or near ultraviolet (UV) on 3, and UV on 4. Land (1969) proposed that the cells of each receptor layer contains a different photopigment. Peaslee and Wilson (1989) examined the spectral sensitivity of the AM eye of *Maevia inclemens* psychophysically based on the oculomotor reflex and reported that jumping spiders are shown to have broad spectral sensitivity extending from the UV (330 nm) to deep red

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(700 nm), with maximum sensitivities in the green and UV regions.

Yamashita and Tateda (1976) reported that the AM eye of the jumping spider *Menemerus confusus* has UV cells with maximum sensitivities at about 360 nm, blue cells at 480–500 nm, green cells at 520–540 nm, and yellow cells at 580 nm. Possession of four types of visual cells suggests that jumping spiders are capable of color discrimination over a wide range of the spectrum. On the other hand, DeVoe (1975) found UV cells (maximum sensitivity at 360 nm), green cells (532 nm) and UV-green cells (both 370 nm and 525 nm) in the AM eye of *Phidippus regius*, but not blue or yellow cells. Blest et al. (1981) also found only UV (maximum is 360 nm) and green (520 nm) cells in the AM eye of *Plexippus validus*. If the AM eye has only UV and green cells, it would be difficult for jumping spiders to discriminate various colors, at least, under illumination of visible light.

In the present study we examined color discrimination ability of jumping spiders by heat-avoidance learning in association with colored papers.

Materials and methods

Jumping spiders *Hasarius adansoni* were used throughout this study. They were collected and maintained individually in glass tubes on a diet of fruit flies. Experiments were carried out at a room temperature of about 28–30 °C.

The experimental arena was cylindrical, about 60 mm in diameter and 4 mm in height, and was covered with a transparent glass sheet (Fig. 1). The height of 4 mm allowed spiders to walk freely on the floor, but not on the wall and the ceiling. When spiders were released into the arena, they usually walked about intermittently. If spiders did not walk in the arena for more than 1 min continuously, experiments were stopped and the data were eliminated. The arena was illuminated from above with a tungsten filament lamp. The illumination intensity at the arena was about 10^4 lx. The floor and the wall of the arena was divided into two semicircles by two different colored papers. The colored papers used in this experiments were blue (B), green (G), yellow (Y), red (R), white (W), gray (Gy) and black (Bk), whose reflectance spectra (measured by Hitachi U-3210 Spectrophotometer, using MgO as reference) are shown in Fig. 2.

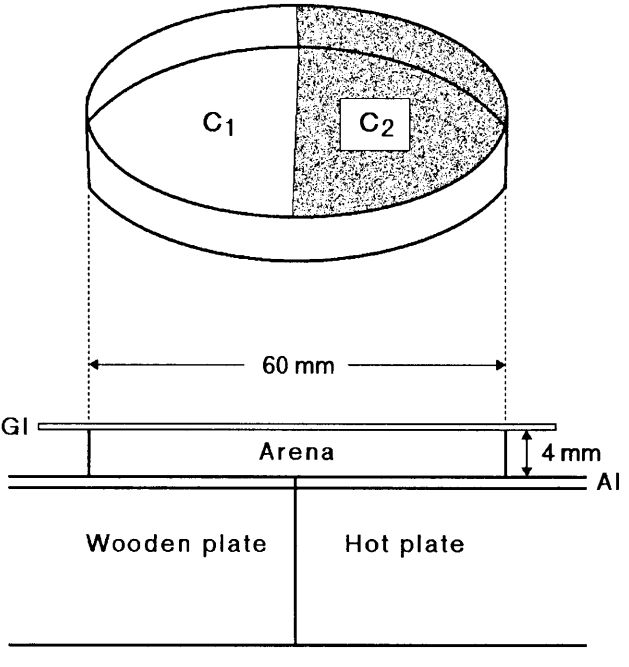

Fig. 1 Diagram of the experimental arena for training. The floor and wall of the arena were divided into two semicircles by two different colored papers (C_1 and C_2). *Gl* glass sheet; *Al* aluminum sheet of about 1 mm thickness; *Lu* lucite sheet of about 1 mm thickness

Usually, a memory experiment for each spider consisted of a pre-training (control, C) session for 3 min, a training session for 3 min, and a post-training (test, T) session for 3 min. The control and test experiments were performed in the same arena, and the training was conducted in an another arena. The test arena was rotated by 90° as compared to the control arena to avoid positional learning due to visual cues outside the arena. Just after the cessation of each training, the spider was moved from the training arena to the test arena. The inter-session interval between the training and the test was about 15 s. In training, one-half of an arena was heated from the bottom by a hot plate through a thin aluminum sheet. The other half of the arena was placed on a wooden heat insulating plate covered with a thin Lucite sheet. Colored papers were attached directly to the aluminum and Lucite sheets. The surface temperature of the colored papers on the hot plate measured with a thermocouple (wire diameter 0.2 mm, Type T) was about 42–44 °C and that on the heat insulating plate was about 30–32 °C. The arena used for the control and the test experiments was always placed on a wooden heat insulating plate covered with a thin Lucite sheet. To remove possible odor cues left by previous spiders in the arena, colored papers were exchanged frequently by new ones, and were wiped gently with cloths with 80% ethanol as frequently as possible.

The choice rate between paired colored papers was defined as $P - N/P + N$, where P is the total time when the spider is present on the heat-associated colored paper, and N is the total time when the spider is present on the another colored paper. The difference between the choice rate during the test session (CRT) and that during the control session (CRC) was used as an index for the memory. Usually four to six different spiders were used for each experiment (e.g., each pair of bars in Fig. 4). Some spiders were used for two or three different experiments at intervals over 2 days.

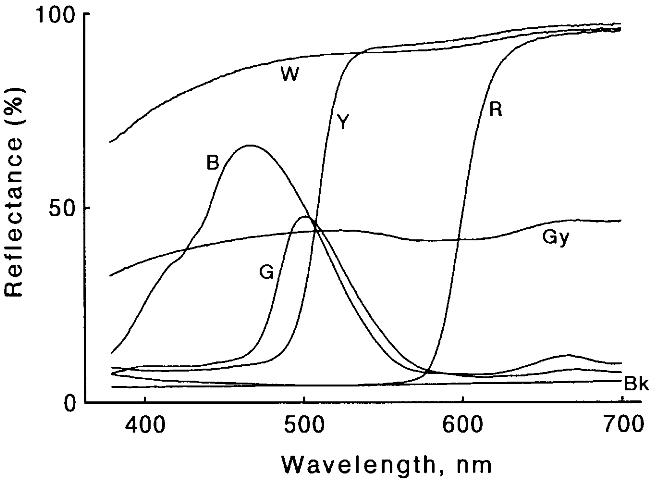

Fig. 2 Reflectance spectra of the colored papers used in the experiments. *B* blue; *G* green; *Y* yellow; *R* red; *W* white; *Gy* gray; *Bk* black

Results

Walking paths in arenas

Figure 3a–c show examples of walking paths of a spider recorded in arenas with a white and black pattern during a control session for 3 min, during a training session for 3 min, and during a test session for 3 min. During the training session, only the black part was heated from the bottom. In the control arena (Fig. 3a), the spider walked both on the black and white parts fairly well. The total time when the spider was present on the black part was 81 s and that on the white part was 99 s, i.e., the choice rate during the control session was −0.10. In the training arena (Fig. 3b), when the spider walked onto the black part, it ran away quickly. The total time when the spider was present on the black part was 3 s and that on the white part was 177 s, i.e., the choice rate during the training session was −0.97. All spiders avoided the heated half well. In most spiders examined, choice rates obtained during the training sessions were about −0.8 or greater negative values in spite of the difference of the two-colored patterns. In the test arena (Fig. 3c), the total time when the spider was present on the black part was 31 s and that on the white part was 149 s, i.e., the choice rate during the test session was −0.66. The mean choice rate obtained from six spiders during the control sessions (mean CRC ± SE) and that during the test sessions (mean CRT ± SE) were −0.08 ± 0.06 and −0.44 ± 0.09, respectively. The mean CRT is significantly different from the mean CRC. The difference between the mean CRT and the mean CRC was −0.36. On the other hand, when the white part of the white and black pattern was heated during the training sessions, the difference between the mean CRT and the mean CRC was −0.41. It is apparent that jumping spiders can learn the black and white pattern.

We have observed that spiders lost their heat-associated color memory within 1 day, i.e., there were no significant differences between choice rates obtained before and 24 h after the trainings.

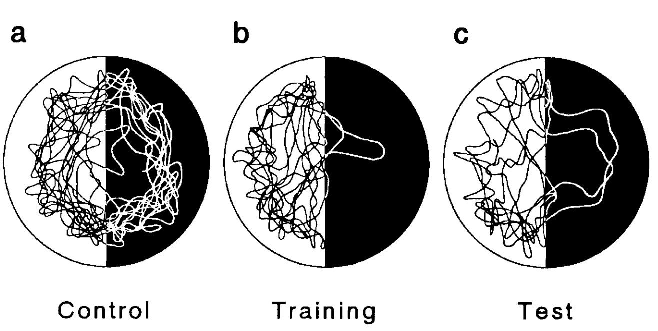

Fig. 3 Walking paths of a jumping spider during **a** pre-training (control), **b** training, and **c** post-training (test) sessions. The animal is video recorded from above using a CCD camera. The duration of each session was 3 min. The control and test experiments were performed in the same arena, and the training was conducted in an another arena. Both arenas had a white and black pattern. In training, only the black part was heated from the bottom by a hot plate

Discrimination among blue, green, yellow, red and gray papers

To examine whether jumping spiders can discriminate color, similar experiments as shown in Fig. 3 were performed with *B*-G, *G*-B, *B*-Y, *Y*-B, *B*-R, *R*-B, *B*-Gy, *Gy*-B, *G*-Y, *Y*-G, *G*-R, *R*-G, *G*-Gy, *Gy*-G, *Y*-R, *R*-Y, *Y*-Gy, *Gy*-Y, *R*-Gy and *Gy*-R patterns, where italic letters indicate heated colored papers during the trainings. Figure 4 shows the mean choice rates during the control sessions (mean CRCs ± SEs) and those during the test sessions (mean CRTs ± SEs). Although the CRC values ranged from +0.16 to −0.08, we did not find any clear tendencies for spiders to choose specific colored papers before training. The mean CRTs were significantly different from the mean CRCs (*t*-test: $P < 0.05$, comparing the mean CRTs and CRCs), except for *B*-Y, *B*-Gy, *G*-R, *Gy*-G and *R*-Gy. The differences between the mean CRTs and the mean CRCs were −0.35 for

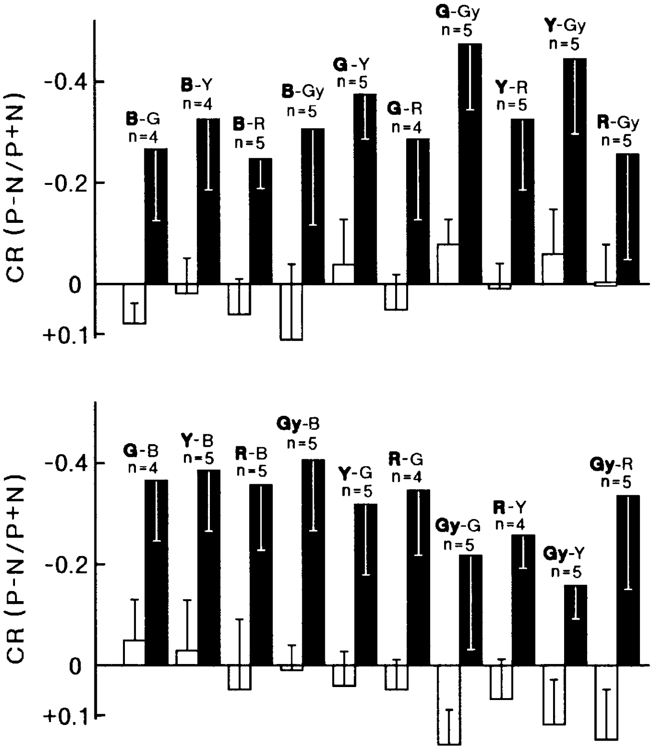

Fig. 4 Discrimination among blue, green, yellow, red and gray papers. Spiders were trained with *B*-G, *G*-B, *B*-Y, *Y*-B, *B*-R, *R*-B, *B*-Gy, *Gy*-B, *G*-Y, *Y*-G, *G*-R, *R*-G, *G*-Gy, *Gy*-G, *Y*-R, *R*-Y, *Y*-Gy, *Gy*-Y, *R*-Gy and *Gy*-R patterns, and tested with the same two-colored patterns. Bold letters indicate heated colored papers during the trainings. Mean choice rates ($CR = P - N/P + N$) during the control sessions (*white bars*) and those during the test sessions (*black bars*) are shown. In this and following figures, negative values are shown upwards. *Vertical lines* show only one side of SE. *n* number of spiders; *B* blue; *G* green; *Y* yellow; *R* red; *Gy* gray

B-G, −0.32 for *G*-B, −0.35 for *B*-Y, −0.36 for *Y*-B, −0.31 for *B*-R, −0.41 for *R*-B, −0.42 for *B*-Gy, −0.42 for *Gy*-B, −0.34 for *G*-Y, −0.36 for *Y*-G, −0.34 for *G*-R, −0.40 for *R*-G, −0.40 for *G*-Gy, −0.38 for *Gy*-G, −0.34 for *Y*-R, −0.33 for *R*-Y, −0.39 for *Y*-Gy, −0.28 for *Gy*-Y, −0.26 for *R*-Gy, and −0.49 for *Gy*-R. All these values are negative. We concluded that jumping spiders can learn blue-green, blue-yellow, blue-red, blue-gray, green-yellow, green-red, green-gray, yellow-red, yellow-gray, and red-gray patterns.

To examine whether the colored papers are discriminated by the hue of colors or the brightness, spiders were trained with *B*-W, *G*-W, *Y*-W and *R*-W patterns, and tested with B-Bk, G-Bk, Y-Bk and R-Bk patterns, respectively. The results are shown in Fig. 5. In this figure, trainings with *R*-W were tried five times, since the effect of the first training with *R*-W was small. The differences between the mean CRTs and the mean CRCs were −0.32 for B-Bk, −0.33 for G-Bk, −0.29 for Y-Bk and −0.27 for R-Bk, where the mean CRT for R-Bk is taken from the fifth test. All these values were within the range shown in Fig. 4. These observations suggest that even after the trainings with *B*-W, *G*-W, *Y*-W and *R*-W patterns, spiders can discriminate the blue, green, yellow and red papers from the black paper. It may be reasonable to conclude that jumping spiders can discriminate the blue, green, yellow and red papers by their hue.

In Fig. 6, spiders were trained five times with *B*-Gy, *G*-Gy, *Y*-Gy, and *R*-Gy patterns. Although the absolute values of the mean CRTs for the fifth tests were larger than those for the first tests in all cases examined, it seems that the effect of repetition in training was small.

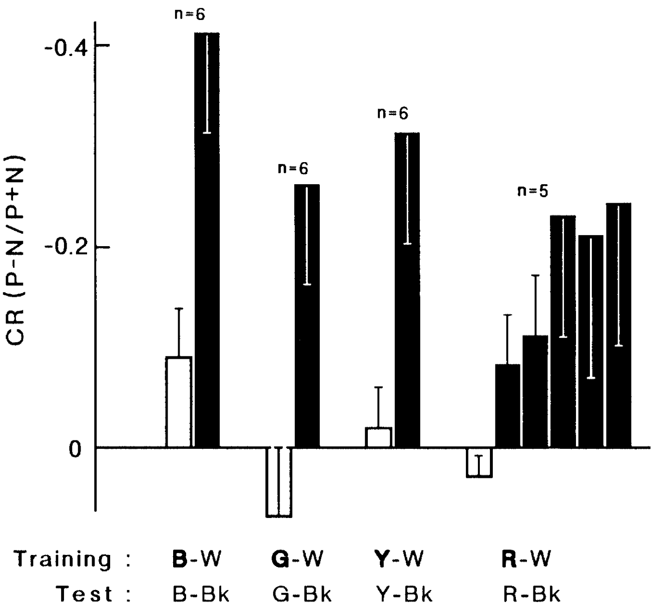

Fig. 5 Effects of brightness of colored papers. Spiders were trained with *B*-W, *G*-W, *Y*-W and *R*-W patterns, and tested with B-Bk, G-Bk, Y-Bk, and *R-Bk* patterns, respectively. Bold letters indicate heated colored papers during the trainings. Trainings with *R*-W and following tests with R-Bk were repeated five times. The interval between the cessation of each test and the start of the next training was about 15 s. *White and black bars* indicate the mean choice rates during the control and test sessions, respectively. *Vertical lines* show only one side of SE. *n* number of spiders; *B* blue; *G* green; *Y* yellow; *R* red; *W* white; *Bk* black

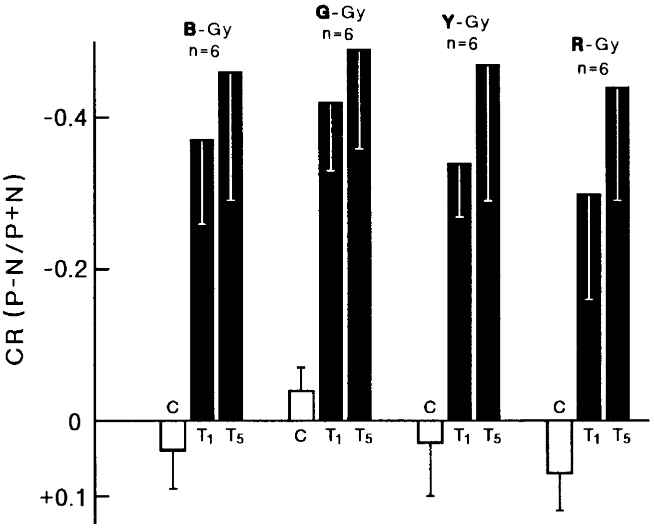

Fig. 6 Effects of repetitive training. Spiders were trained with *B*-Gy, *G*-Gy, *Y*-Gy, and *R*-Gy patterns, and tested with the same two-colored patterns. For each two-colored pattern, trainings and the following tests were repeated five times. The interval between the cessation of each test and the start of the next training was about 15 s. Bold letters indicate heated colored papers during the trainings. The mean choice rates during the control (C), and the first (T_1) and fifth (T_5) test sessions are shown. *Vertical lines* show only one side of SE. *n* number of spiders; *B* blue; *G* green; *Y* yellow; *R* red; *Gy* gray

Discussion

In the present study, we found that the jumping spider *H. adansoni* can be trained to avoid colors which are associated with high temperature, although the biological meaning of the heat-avoidance learning in association with color is not yet known. We have observed similar heat-avoidance learning in association with colored paper in the jumping spiders *M. confusus* and *Pl. paykulli* (data not shown). Since jumping spiders eat only living prey such as fruit flies, it is difficult to perform learning experiments in association with food. Therefore, heat-avoidance learning seems to be an effective method to examine the color discrimination ability of jumping spiders.

True color vision has been defined as the ability of an animal to discriminate colors based on the spectral composition of the targets, regardless of their brightness (Menzel 1979). Convincing demonstrations of true color vision in invertebrates by behavioral experiments have been achieved in the honeybee *Apis mellifera* (for a review see Menzel and Backhaus 1989), the blowfly *Lucilia cuprina* (Fukushi 1989), the mantis shrimp *Odontodactylus scyllarus* (Marshall et al. 1996), the swallowtail butterflies *Papilio xuthus* (Kinoshita et al. 1999) and *P. aegeus* (Kelber and Pfaff 1999), and the hummingbird hawkmoth *Macroglossum stellatarum* (Kelber and Henique 1999). Here we have demonstrated that jumping spiders could discriminate blue, green, yellow, and red papers from each other. Moreover, since spiders trained with blue-white, green-white, yellow-white, and red-white patterns could discriminate blue, green, yellow, and red from black, colored paper seems to be discriminated mainly by its hue. It is apparent that jumping spiders have some form of true color vision.

The utilization of wavelength information can be behaviorally dependent. For example, honeybees have color vision when they are searching for flowers, but are color blind in some other cases (for a review see Menzel and Backhaus 1989). However, it is not yet known whether the color vision of jumping spiders is behaviorally dependent or not.

For true color vision, at least two spectrally different types of visual cells with overlapping spectral sensitivity are required. DeVoe (1975) found that the AM eye of the jumping spider *Ph. regius* has UV and green cells. Subsequently, Blest et al. (1981) also found UV and green cells in the AM eye of *Pl. validus*. The spectral sensitivity curves reported by DeVoe (1975) and Blest et al. (1981) seem to show that the sensitivity of the UV mechanism does not extend further than 450 nm. If the AM eye has only UV and green cells, wavelengths greater than 450 nm must be detected solely by green cells, making it very difficult for jumping spiders to discriminate yellow and red from each other. In the present study, we showed that jumping spiders could discriminate yellow and red papers from each other. However, the yellow paper used in this study was somewhat reflectant around 450 nm, while the red paper was not (Fig. 2). Therefore, there remains the possibility that jumping spiders might discriminate the yellow and red papers with UV and green cells. We also showed that jumping spiders trained with red-white patterns could discriminate the red from black, indicating that the red paper is discriminated mainly by its hue, which would require another type of visual cell other than UV and green cells.

Yamashita and Tateda (1976) reported that the AM eye of the jumping spider *M. confusus* has UV, blue, green and yellow cells. The spectral sensitivities of the blue, green and yellow cells overlap each other between 420 and 620 nm. If just two types of the visual cells took part in the color vision of jumping spiders, it would be possible for them to discriminate the blue, green, yellow and red papers used in this study mainly by their hue. Therefore, at this stage, we can not tell if the color vision of jumping spiders is dichromatic based on two types of visual cells out of the blue, green, and yellow cells, trichromatic based on two types of visual cells out of blue, green, and yellow cells, and UV cells, or tetrachromatic based on UV, blue, green, and yellow cells.

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