



Contribution of a visual pigment absorption spectrum to a visual function: depth perception in a jumping spider

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Absorption spectra of visual pigments are adaptively tuned to optimize informational capacity in most visual systems. Our recent investigation of the eyes of the jumping spider reveals an apparent exception: the absorption characteristics of a visual pigment cause defocusing of the image, reducing visual acuity generally in a part of the retina. However, the amount of defocus can theoretically provide a quantitative indication of the distance of an object. Therefore, we proposed a novel mechanism for depth perception in jumping spiders based on image defocus. Behavioral experiments revealed that the depth perception of the spider depended on the wavelength of the ambient light, which affects the amount of defocus because of chromatic aberration of the lens. This wavelength effect on depth perception was in close agreement with theoretical predictions based on our hypothesis. These data strongly support the hypothesis that the depth perception mechanism of jumping spiders is based on image defocus.

Key words: opsin, vision, defocus

Opsin-based pigments (including visual pigments) consist of opsin, a protein moiety, and retinal, a chromophore. Functional diversity of pigments with different absorption spectra supports various visual functions in animals. Well-known examples are opsin-based pigments with absorption

characteristics optimized to ambient electromagnetic spectra, which contribute to vision in dim light¹. A repertoire of opsin-based pigments with different absorption maxima also forms the molecular basis of color vision or wavelength discrimination. In this review, we discuss the first known example of a visual pigment's absorption characteristics contributing to depth perception, i.e., the ability to visually judge the distance of an object, in a jumping spider (Fig. 1a)².

Image defocus generated by a green-sensitive visual pigment

In ordinary camera-type eyes (including human eyes) photoreceptor cells containing visual pigments are distributed two-dimensionally across the retina. In contrast, the main eyes of jumping spiders, referred to as principal eyes (PEs), have a three-dimensionally structured retina containing four tiered layers of rhabdomeres, which are the photoreceptive portions of photoreceptor cells (Fig. 1)^{3,4}. The lens of the PE has a long focal length giving high visual acuity, comparable to that of vertebrate eyes^{4,5}. As a consequence of this long focal length, the lens generates considerable chromatic aberration, whereby its focal length differs according to the wavelength of the transmitted light (Fig. 1b). Therefore, the depth in the retina at which the image is focused depends not only on the distance of the object but also on the wavelength of light^{4,6}. It has been hypothesized that the photoreceptor layers contain different visual pigments such that each is sensitive to the wavelength of light that focuses on that layer^{4,6}. We found that Rh1, a green-sensitive visual pigment, is localized in Layers 1 and 2, the

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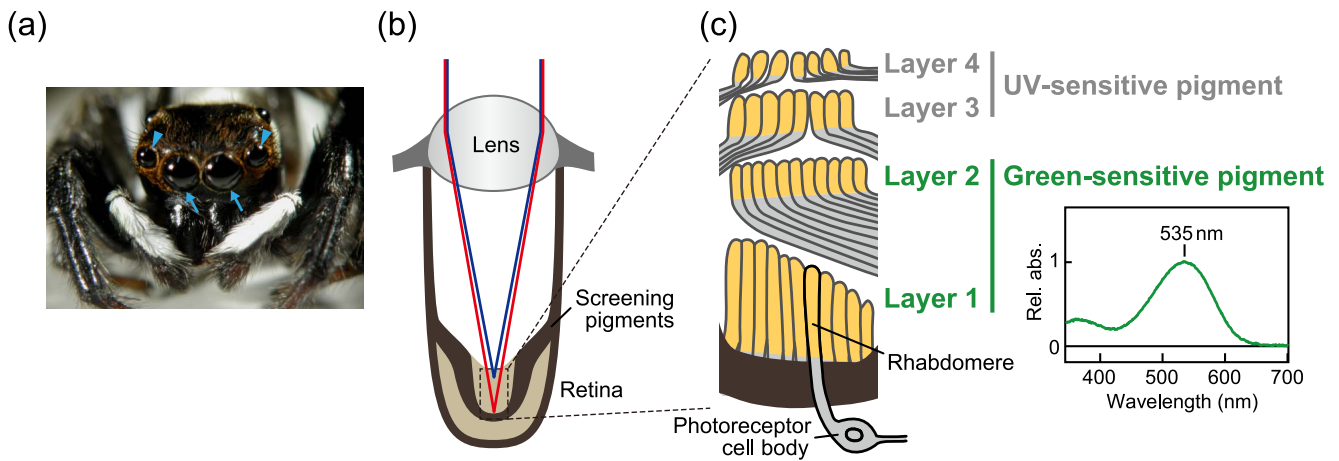


Figure 1 (a) Frontal view of the jumping spider *Hasarius adansoni*. Principal eyes (PEs; arrows) and anterior lateral eyes (ALEs; arrow heads) are indicated. (b) Schematic drawing of the structure of the principal eye, which is a camera-type eye with a lens projecting onto a retina. Long wavelength light (red line) has a longer focal length than shorter wavelength light (blue line) because of chromatic aberration of the lens. (c) *Left*, Schematic drawing of a horizontal section of the PE retina. The photoreceptor layers consist of rhabdomeres (yellow) of photoreceptor cells. The cell body of a photoreceptor cell is shown as an example. *Right*, The visual pigments localized in the four layers and the absorption spectrum of the green-sensitive visual pigment.

two deepest layers (Fig. 1c), indicating that these layers receive green-light images. However, it has been reported that green light focuses on Layer 1 but not on Layer 2⁶, suggesting that the latter layer always receives a defocused image. Jumping spiders have another visual pigment that maximally absorbs blue light (which is focused on Layer 2), but this pigment has never been detected in the PEs². This implies that the defocus of images received by Layer 2 may be biologically relevant.

Hypothesis of a depth perception mechanism based on image defocus

Pentland⁷ has reported that the amount of image defocus, which depends on the distance between a lens and an object, can in principle be used to unambiguously determine visual depth. Although Pentland showed that humans use image defocus for a rough estimation of the relative depths of objects⁷, to date no animals have been reported to use image defocus as an absolute depth cue. We tested the possibility that the image defocus in Layer 2 is involved in depth perception because, in principle, the distance to an object can be absolutely determined from the amount of defocus in Layer 2 (Fig. 2a).

Depth perception is crucial for jumping spiders because they visually judge the distance to their prey and then jump accurately onto the target. Although it has been reported that the PEs and other forward-facing eyes, the anterior lateral eyes (ALEs, see Fig. 1a), are involved in depth perception⁸, the mechanism by which they perform this function is unknown. We found that just one PE was sufficient for accurate jumps (Fig. 3), demonstrating that the PEs are capable of monocular depth perception. To date, accommo-

dation (i.e., focal adjustment)^{9,10} and motion parallax (i.e., image motion on the retina)¹¹ are the only known mechanisms of absolute monocular depth perception in animals. Some insects obtain motion parallax information by making side-to-side translational movements of the head. However, no motion that could generate significant parallax was observed prior to jumps in the behavioral experiment, and PEs have no focal adjustment mechanism¹², implying that a different monocular mechanism is responsible for depth perception. Therefore, we hypothesized that jumping spiders perceive depth on the basis of the amount of defocus in images received by Layer 2 (Fig. 2a).

Behavioral test of the hypothesis using green and red light

The hypothesis predicts that jumping spiders would underestimate target distance under red ambient light because of chromatic aberration of the lens. Chromatic aberration results in a longer focal length under red light than green light^{2,6}, and thus the defocus of an object under red light is equal to that generated by a closer object under green light (Fig. 2b). If jumping spiders judge distance on the basis of the distance–defocus relationship of green light, which Rh1 absorbs maximally under natural light conditions, then they would underestimate the distance and therefore make shorter jumps under red illumination. This prediction was tested using spiders with their bilateral ALEs occluded under monochromatic green (approximately 520 nm) and red (approximately 630 nm) light, to which only Layers 1 and 2 are sensitive. Jump distances were accurate under green light but significantly shorter than the actual target distances under red light (Fig. 4a). In the “1 × red” condition, the intensities

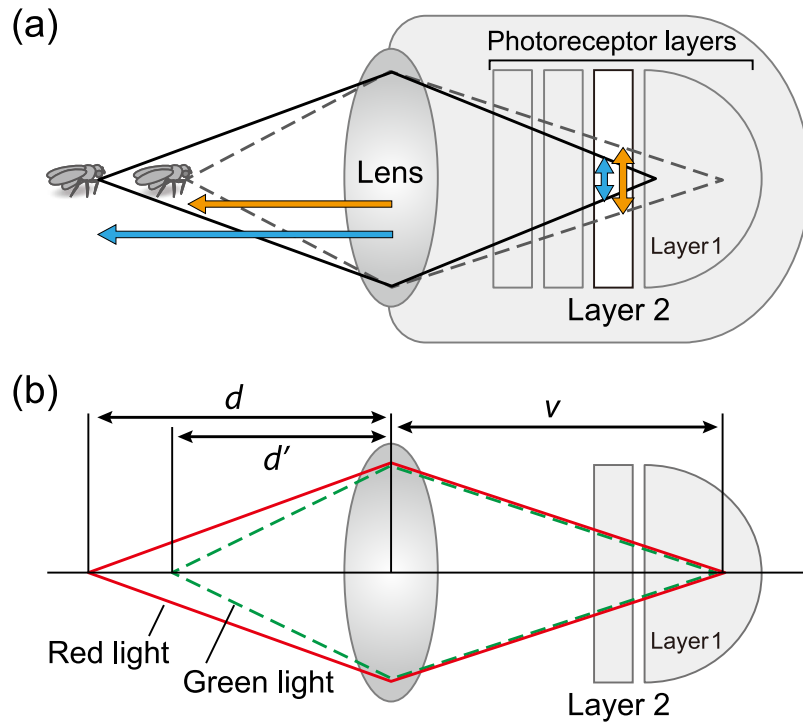


Figure 2 (a) Schematic drawing of the light from two targets (solid and broken lines) passing through the lens and being received by Layers 1 and 2. The amount of defocus at Layer 2 (double-headed arrows) depends on the distance between the lens and the objects (arrows). (b) The same amount of defocus at Layer 2 is generated by red light from a point at distance d (red solid line) and green light from a closer point at distance d' (green broken line), both of which are focused at the same point (distance v) in Layer 1 of the retina.

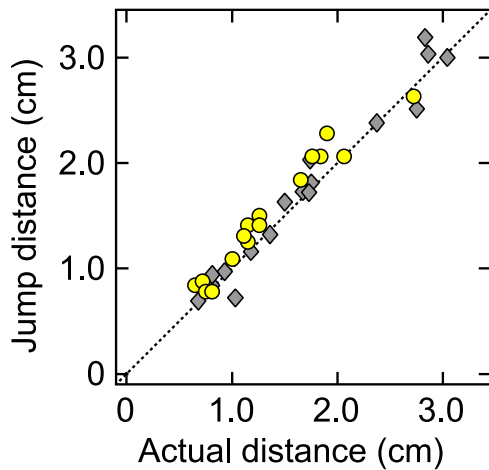


Figure 3 Jump distance versus the actual target distance for normal jumping spiders (gray diamonds) and spiders with one PE and both ALEs occluded (yellow circles).

of the green and red lights were adjusted using purified Rh1 pigment so that the two types of light activated Rh1 equally. Under those conditions, the subjective brightness of the two lights for the spider should be identical. In addition, the ratio of the jump distance to the actual target distance under a 6-fold brighter red light ($6 \times$ red) was similar to the ratio

under the $1 \times$ red light (Fig. 4a). Therefore, the shortening of jump distances under red ambient light can be attributed to the change of wavelength rather than intensity.

Theoretical calculation of jump distances under red light

To evaluate these results, we calculated the theoretically predicted jump distances based on a simple optical model. Figure 2b shows rays of red light from a point at distance d and green light from another point at distance d' , both generating the same amount of defocus at Layer 2. If the hypothesis is correct, d' corresponds to the distance estimated by the spiders, i.e. the jump distance. The lens equations for the red and green light are as follows:

$$\frac{1}{d} + \frac{1}{v} = \frac{1}{F_r}$$

$$\frac{1}{d'} + \frac{1}{v} = \frac{1}{F_g},$$

where v is the distance between the lens and the focal point, and F_r and F_g are the focal lengths of the lens for red and green light, respectively. From these equations, we can obtain

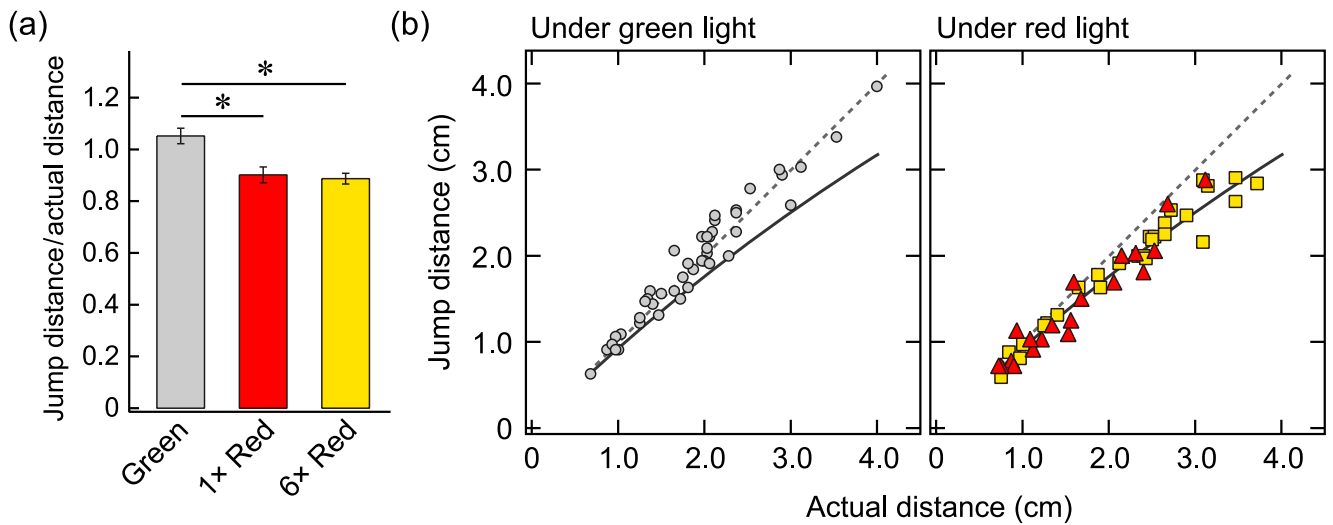


Figure 4 Behavioral evidence for depth perception from image defocus in jumping spiders. (a) Ratios of the jump distance to the actual distance. Data are presented as the mean \pm s.e.m ($n=4$). * $P<0.05$. (b) Jump distances versus actual distances under green light (gray circles), $1 \times$ red light (red triangles) and $6 \times$ red light (yellow squares). The theoretical curves under red light (black solid curves) and green light (gray broken curves) are also shown (see text for explanation).

$$d' = \frac{1}{f} \left(1 - \frac{1}{1+fd} \right),$$

where

$$f = \frac{1}{F_g} - \frac{1}{F_r}.$$

We experimentally determined F_g and F_r using an optical method^{4,6}. The obtained theoretical curves of d' correspond well with the observed jump distances (Fig. 4b). These results strongly support the hypothesis that depth perception in jumping spiders is based on the amount of defocus in the images received by Layer 2.

Further questions

Rh1 is also likely to be used for receiving focused images in Layer 1 because this layer has a wide focus range⁶. In optical theory, to accurately determine the amount of defocus in an image, it is important to compare focused and defocused images of the same object¹³. We found that Layer 1 had the same spectral sensitivity as Layer 2 and that spiders jumped accurately under green light, to which only Layers 1 and 2 are sensitive. These results support an idea that images received by Layers 1 and 2 are compared in order to extract the depth information. At present, however, there is no direct evidence of this operation taking place. Further studies are needed to elucidate whether and how such mechanisms are neurally implemented for the purpose of depth perception.

Our study also revealed that an ultraviolet (UV)-sensitive visual pigment is localized in Layers 3 and 4 (Fig. 1c)², where UV is focused⁶, indicating that jumping spiders could

receive focused images both under UV and green light. In addition, previous behavioral studies have suggested that jumping spiders discriminate between UV and visible light^{14,15}. Therefore, compensation for chromatic aberration by the combination of retinal structure and absorption characteristics of the pigments could enable wavelength discrimination in jumping spiders. In contrast, some vertebrate eyes have a multi-focal lens to compensate for chromatic aberration¹⁶. The differences in physiological roles of the two UV-sensitive layers remain unclear.

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