

Correspondence

Spectral filtering enables trichromatic vision in colorful jumping spiders

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Jumping spiders (family Salticidae) are masters of miniature vision, achieving higher spatial resolution in relation to body size than any other animal [1]. While most members of this family do not use color in intraspecific communication, several genera serve as emerging examples of rapid evolutionary radiation in sexual display coloration [2]. These include the Australasian *Maratus* ‘peacock’ spiders, and the American genus *Habronattus*. Males of these genera are often brilliantly colored on body surfaces they showcase to females during elaborate courtship dances (Figure 1A). However, molecular and electrophysiological data suggest that color vision in the acute ‘principal’ eyes of most jumping spiders is based on only two types of photosensitive pigment, one sensitive to ultraviolet (UV) light, the other to green light [3–5]. We report here that *Habronattus* jumping spiders may achieve substantially better color vision via a mechanism previously unknown in spiders: the shifting of sensitivity of a subset of their photoreceptors from green to red via a long-pass filter positioned in their retina. Trichromatic vision resulting from this filter system should markedly enrich these animals’ perception of color, including reds, oranges and yellows often found in their courtship displays.

Like all salticids, *Habronattus* has a modular visual system formed by four specialized eye pairs. The large principal eyes serve spatial vision and also support color vision. Their small retinas have a characteristic boomerang shape (Figure 1B,C), and are composed of four photoreceptor tiers (Figure 1D–F) [6]. In salticids with known principal retina sensitivities, the two proximal tiers (1 and 2) consist

of a dense, regular mosaic of green-sensitive photoreceptors, while the two distal tiers (3 and 4) are UV-sensitive with a less organized mosaic structure [4,5].

We investigated the sensitivity of the photoreceptors in the principal eye retinas of *Habronattus pyrrihrix*, a species in which males display green, cream, orange, and red ornaments to females during complex courtship sequences (see Supplemental Movie S1). Previous research has established that long-wavelength colors

play a role in foraging and courtship [7,8]. However, the dichromatic vision of closely related species would provide limited color perception for short wavelength colors, and no ability to discriminate long wavelength colors. Such poor spectral discrimination seems mismatched to the diverse color palette of *H. pyrrihrix* and its congeners.

Using spectrophotometric techniques, we determined transmittance of optical structures

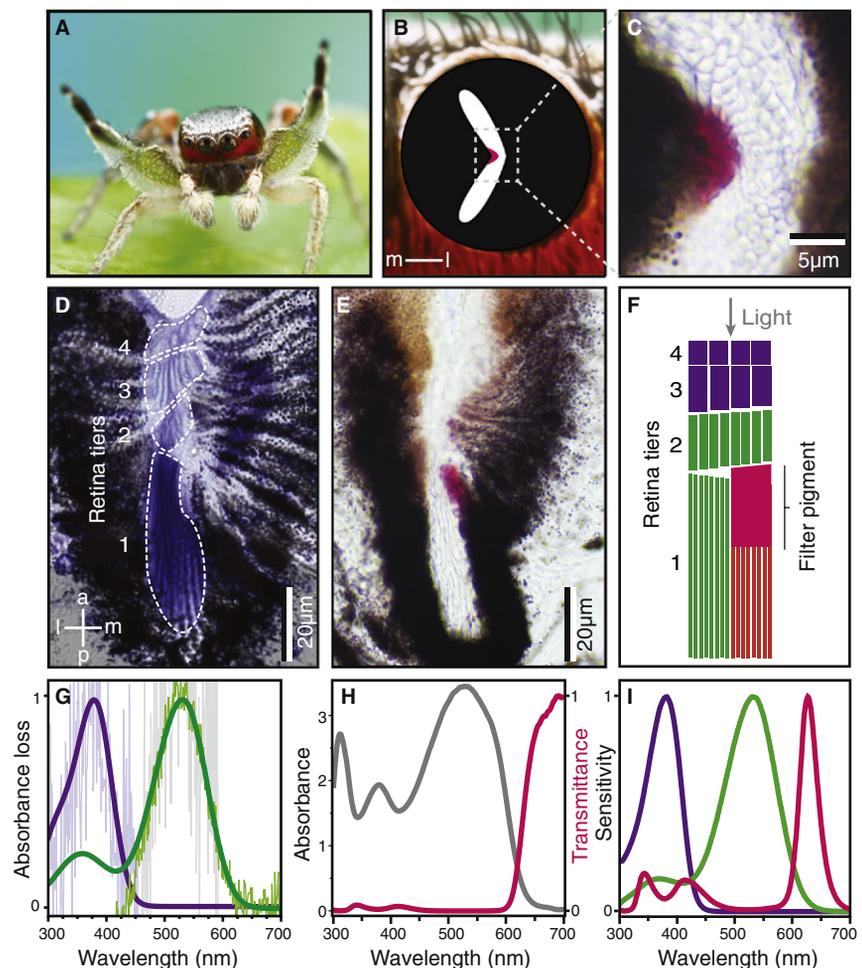


Figure 1. Spectral filtering of *Habronattus pyrrihrix* photoreceptors.

(A) Courtship display of male *H. pyrrihrix* (photo: Colin Hutton). (B) Boomerang-shaped retinas superimposed on principal eye lenses, small red area indicates filter position. (C) Red filter pigment visible in unstained coronal cryosection (14 μm thick) of the principal retina. (D) Tiered retina structure in thin horizontal section of principal retina: a, anterior; p, posterior; l, lateral; m, medial. (E) Red filter pigment visible in unstained horizontal cryosection (14 μm thick) of the principal retina. (F) Diagram of tiered arrangement of green and UV-sensitive photoreceptors in longitudinal view of principal retina center of *H. pyrrihrix*. Red area indicates long-pass filter pigment, shifting sensitivity of tier 1 photoreceptors from green to red. (G) Normalized visual pigment absorbance difference spectra in proximal (green, $n = 11$) and distal (violet, $n = 2$) retinal tiers with best-fit templates. (H) Absorbance (grey) and transmittance (red) of filter at maximal observed thickness ($n = 6$). (I) Predicted sensitivity of three photoreceptor classes, UV (377 nm), green (530 nm), red (626 nm), after light filtering by optics and filter pigment. All axes in G–I are linear.

in the light path (Supplemental Results), and measured absorbance spectra of visual pigments in unfixed, cryosectioned retinas. Consistent with previous work, we found two photopigments (Figure 1G), one green-sensitive (tiers 1 and 2, $\lambda_{\max} = 530$ nm) and one UV-sensitive (tiers 3 and 4, $\lambda_{\max} = 377$ nm). UV-sensitive photoreceptors exhibited a second peak at ~ 530 nm, similar to the double-peaked UV-green photoreceptors described previously in other species [3]. In addition, we found an unexpected, ruby-red photostable pigment positioned in the light path of a population of foveal tier 1 rhabdoms (Figures 1C–F and Supplemental Figure S1B,C). The pigment is found in both sexes, and functions as a spectral long-pass filter (Figure 1H) that allows only red light to reach the underlying green-sensitive photoreceptors. This would produce a large shift of their λ_{\max} from 530 nm to 626 nm, making this spatially acute part of tier 1 specifically sensitive to red light. As a result, these now ‘red’ photoreceptors provide a third color channel (Figure 1I). Another unique feature of this filter system is its restriction to a small region of the retinal center, which results in a trichromatic area surrounded by an otherwise dichromatic retinal field.

In most eyes where they occur, spectral filters function to sharpen the sensitivity peaks of their associated photoreceptors, but members of some taxa, such as butterflies and stomatopods, multiply spectral sensitivity using filtering [9]. This strategy comes with a decrease in sensitivity of filtered photoreceptors. Consistent with this, a recent study of *H. pyrithrix* shows that behavioral responses to color stimuli weaken under dim light [8]. However, the bright habitats typical of *Habronattus* species should allow filter-based trichromacy to functionally increase the discriminable color gamut, thereby aiding color-based prey selection and female assessment of colorful male courtship displays. We simulated perceived contrasts of four male color ornaments using a dichromatic (UV, green) and a trichromatic (UV, green, red) model of the *H. pyrithrix* visual system. Compared to a dichromatic system, adding a third, red-sensitive receptor increases discriminability of long

wavelength colors (for example, it more than doubles the perceptual distance between green 1st legs and orange 3rd legs; see Supplemental Figure S2). However, because of the spatial extent of the filter, only a small area in the center of the visual field of each eye is trichromatic. This suggests that complex male displays may present specific challenges for female color vision, and that the gaze movements observed in salticids [10] may play a role in extracting spectral information from the visual scene.

Is filter-based trichromacy specific to *H. pyrithrix* limited to certain genera that particularly profit from increased color discrimination, or common to all jumping spiders? We found similar filters in four other *Habronattus* species selected from across the *Habronattus* phylogeny (*H. conjunctus*, *H. dossenus*, *H. virgulatus*, *H. hirsutus*; Figure S1A). Thus, filter-based trichromacy may be a general feature of this genus. However, such filters are absent in other salticid genera we sampled, such as *Salticus* and *Phidippus*, which do not use color ornaments during courtship. This suggests that a shift from dichromacy to trichromacy may have played an important role in the evolution of the distinctively colorful courtship displays of *Habronattus* jumping spiders. Future studies will examine if improved color discrimination ability, conveyed by intraretinal filtering, represents a key innovation that enabled the extensive radiation and success of the genus *Habronattus*.

In conclusion, our study offers a solution to the long-standing puzzle of how some salticids see color and opens the door for future studies on co-evolution of color vision and coloration. Future work should focus on the taxonomic extent of this filter-based trichromacy, as well as the adaptive benefits most likely to have favored its evolution. In particular, we suggest that trichromatic species may realize significant advantages when foraging in prey communities that include red and yellow aposematic prey.

SUPPLEMENTAL INFORMATION

Supplemental information contains experimental procedures and supplemental results, two figures, and one movie and can be found with this article online at <http://dx.doi.org/10.1016/j.cub.2015.03.033>.

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Supplemental Information: Spectral filtering enables trichromatic vision in colorful jumping spiders

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Supplemental Results

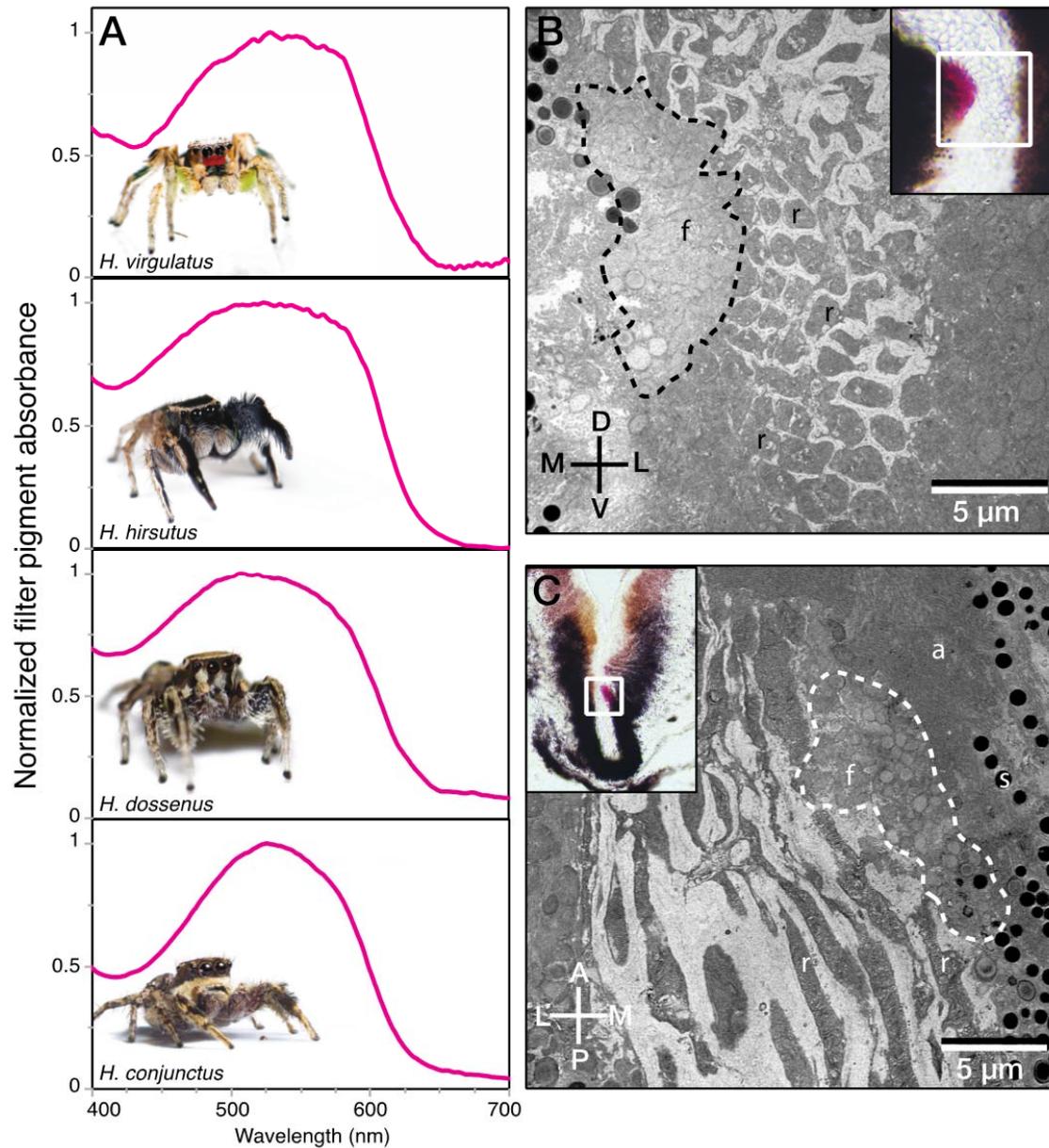


Figure S1. Supplemental filter pigment data

(A) Normalized absorbance of filter pigments in four *Habronattus* species (n = 1 male per species)
 (B,C) Electron micrographs of retina sections containing filter pigments in *H. pyrrihrix*. Pigment is contained in vacuoles distal to tier 1 photoreceptors on the medial side of the retina, placing it in their light path; f: filter pigment, r: rhabdom, a: axons of distal tier photoreceptors, s: screening pigment granule (B) Frontal section, D: dorsal, V: ventral, M: medial, L: lateral, (C) Horizontal section, A: anterior, P: posterior, L: lateral, M:

medial.

Transmittance of lens and vitreous media

The corneal lens of *H. pyrithrix*' principal eye has high transmittance at long wavelengths, increasing gradually from shorter wavelengths with 50% transmittance (T50) at 320 nm, placing it in lens class II as defined by Hu et al. [S1]. The vitreous medium is more transparent throughout the measured spectrum (T50 = 296 nm), while showing a similar trend to the lens of gradually increasing transmittance above 300 nm.

Effect of shifted sensitivity on discriminability of male ornaments

We simulated discriminability of four male color ornaments (Figure S2A) to a dichromatic (UV and green) and a trichromatic (UV, green, red) color opponency model of *H. pyrithrix*' visual system. Compared to the dichromatic model (Figure S2B), trichromacy increases the perceptual distances between all pairs of male color ornaments (Figure S2C). When comparing these estimated perceptual distances to a commonly used discriminability threshold of 2.3 derived from behavioral work in bees [S2], we find that these increased perceptual distances under trichromacy may allow females to reliably discriminate most male display elements based on color alone. The highest increases are predicted for contrasts between first leg pair and face, pedipalps and third leg pair (Figure S2C).

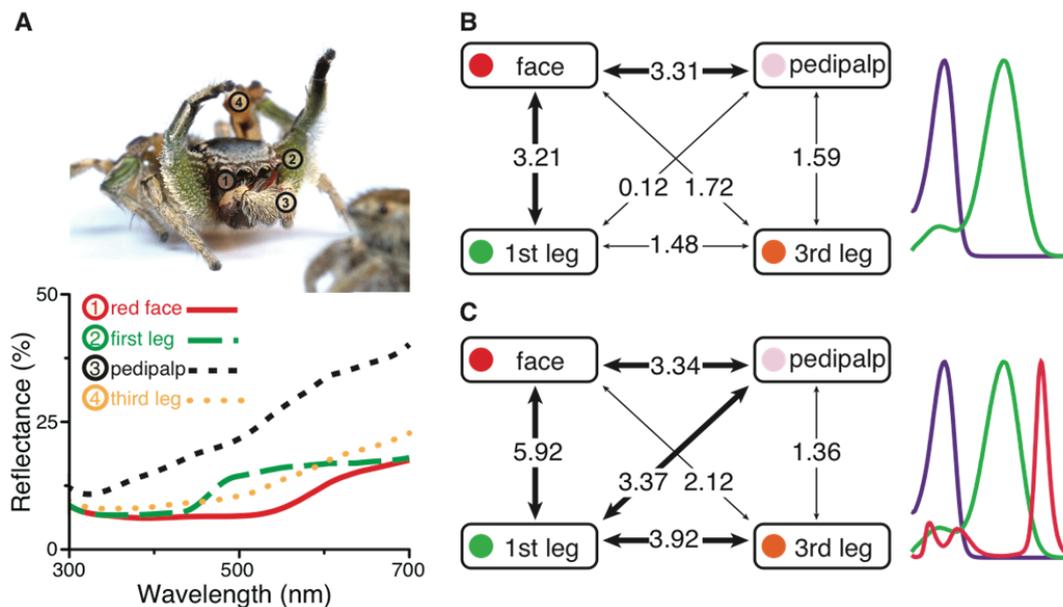


Figure S2. Discriminability of male color ornaments

(A) Reflectance of color patches of male *Habronattus pyrithrix*, displayed during courtship.

(B,C) Perceptual distances (ΔS^1) of male ornament patch contrasts for di- and trichromatic models, assuming a Weber fraction of 0.05. Numbers in bold are higher than the standard behavioral discrimination threshold of 2.3, determined in Hymenoptera [11] (B) dichromatic model incorporating only UV and green photoreceptors, (C) trichromatic model additionally including a sub-population of red-shifted green photoreceptors shows an increase in discriminability of most color patches.

Supplemental Experimental Procedures

Animals

Habronattus pyrrithrix Chamberlin 1924 (Salticidae: Pelleninae) is a sexually dimorphic jumping spider with colorful males and drab females, found in riparian habitats from southern California and Arizona, USA, to Sinaloa, Mexico [S3]. We used adult spiders caught in Queen Creek, AZ (33°13'29"N, 111°35'34"W) in May 2014, as well as lab-raised offspring of spiders caught in the same field site the previous year. Spiders were kept in individual plastic containers (5.5 cm tall and 2.5 cm in diameter) in a climate-controlled room (24°C, 60% RH, 16:8 light:dark photoperiod). We fed adult spiders two times a week with 3-5 one-week-old crickets; spiderlings were fed *ad libitum* with newly-hatched pinhead crickets and *Drosophila melanogaster*.

Ocular media transmittance

Lens transmission was measured by excising lenses and immediately measuring transmission through the central lens axis with a microspectrophotometer (MSP) at 1 nm increments from 300-800 nm (20/20 PVTM Microspectrophotometer, CRAIC Technologies, San Dimas, CA). Vitreous media was extracted by inserting a microsyringe (1700 Series gas-tight syringe with custom needle, Hamilton Company, Reno, NV) through the soft cornea of the PE, and removing ca. 2 μ l of fluid vitreous humor from each eye tube. Vitreous humor transmittance was then measured in 1 nm increments from 200-800 nm using a micro-volume plate reader (Take3 Micro-Volume plate and Epoch Microplate Spectrophotometer, BioTek Instruments, Inc., Winooski, VT), using a 500 μ m light path, which is a typical eye tube length of *H. pyrrithrix*. Vitreous humor transmission spectra were compared with haemolymph transmission spectra, which are qualitatively distinct, to ensure that vitreous humor extractions had not breached the wall of the eye tube and become contaminated with haemolymph.

Photoreceptor absorbance

Spiders were dark-adapted for at least 12 hours prior to sectioning. We then performed the following steps under dim red lighting. Spiders were chilled, and their legs and abdomen quickly removed. The cephalothorax was then embedded in O.C.T. compound medium (Scigen Scientific, Gardena, CA) and flash-frozen with cryogenic spray. We cryosectioned at -18°C in the coronal plane, using a section thickness of 14 μ m. Retinal layers were identified from anatomical landmarks, including their distance from the pit lens, rhabdom morphology and position within the retinal boomerang shape. Cross-sections were sandwiched between two glass cover slips (Fisherfinest® Premium Cover Glass, Thermo Fisher Scientific, Inc., Waltham, MA) and suspended in either mineral oil or basic insect Ringer's solution (pH \approx 7.5, [S4]) contained by a ring of silicon grease. We verified that absorbance profiles of photopigments were identical between these two media. However, mineral oil provided higher tissue stability, particularly for measurements in the UV. We therefore report results measured in mineral oil here. Using a custom-built, single-beam MSP [S5, S6], we measured transmission of retinal cells using a ca. 3 μ m beam diameter. Retinal tiers I and II were scanned at 1 nm increments over a wavelength range from 400-800 nm. For scanning of tiers III and IV, we used a Zeiss 32x Ultrafluar objective (Carl Zeiss AG, Oberkochen, Germany) and measured from 300-700 nm. We scanned an empty region of the slide prep as reference, and then performed an initial scan of focal photoreceptors. This retinal region was then photobleached with 2 minutes of exposure to white light and re-scanned. We subtracted absorbance spectra of bleached regions from the first scan, the difference spectra representing photoreceptor absorbance loss. These difference spectra of each photoreceptor were then de-trended by fitting a line to the long wavelength tail of the spectrum and subtracting it from the curve. Profiles were then averaged and fitted with visual pigment templates [S7].

Intra-retinal photostable pigment transmittance

Unstained cryosections viewed under white light revealed a distinct, photostable red pigment between tiers I and II in the acute zone of the retina. To characterize the positioning and transmission profile of this retinal filter pigment, we cryosectioned spiders under normal room lighting using the same sectioning approach described above. In all series of frontal sections, red

filter pigment was readily apparent via light microscopy in 2-3 successive 14 μm thick sections. Sections from *H. pyrrithrix* (3 females, 3 males) were suspended in mineral oil and filter absorbance measured at 1 nm increments either from 400-800nm as described above, or from 300-800 nm in a different MSP (20/20 PV™ Microspectrophotometer, CRAIC Technologies, San Dimas, CA). As filter optical density varied with section depth, we normalized all absorbance spectra to the spectrum with the highest observed optical density, because these sections were most likely to represent those with pigmentation extending the full 14 μm section thickness.

We verified the presence of long-wavelength filter pigments in single individuals of four additional *Habronattus* species (*H. virgulatus*, *H. hirsutus*, *H. dossenus*, *H. conjunctus*). These species represent phylogenetically diverse species groups within the genus [2]. Preparation and measurements were as described for *H. pyrrithrix* above, but only filter pigment spectra were measured. Additionally, members of other salticid genera, *Phidippus audax* (Salticidae: Dendryphantinae) and *Salticus scenicus* (Salticidae: Salticinae), were examined for presence of intraretinal filter pigments (n = 3 individuals sampled per species). These latter two species were found to lack any measurable retinal filters.

Histology

Light microscopy: Spider cephalothoraxes were fixed in cold 2.5% glutaraldehyde in 0.1 M PBS. The specimens were rinsed in PBS, post-fixed in 1% osmium tetroxide with 1% potassium ferricyanide, rinsed in PBS again, dehydrated through a graded series of ethanol and embedded in Epon. Semi-thin (300 nm) sections were cut on a Reichert-Jung Ultracut Microtome, and stained with 0.5% toluidine Blue for light microscopic imaging.

Electron microscopy: We first cryosectioned cephalothoraxes in horizontal or coronal orientation at 14 μm thickness as described above. Sections were picked up on microscope slides, inspected for red filter pigment and suspended in 2.5% glutaraldehyde. Following light microscopic imaging, sections were rinsed in PBS, post-fixed in 1% osmium tetroxide with 1% potassium ferricyanide, rinsed in PBS again, dehydrated through a graded series of ethanol and embedded in Epon on the microscope slide. Samples embedded on the slides were alternately placed in boiling water and liquid nitrogen to cause the Epon block containing the thick cryosection to separate from the glass slide. Ultrathin sections (75 nm) were then cut on a Reichert-Jung Ultracut Microtome, stained with uranyl acetate and Reynold's lead citrate, examined and imaged on a Jeol JEM-1011 transmission electron microscope with Hamamatsu ORCA-HR digital camera using an AMT image capture system.

Modeled photoreceptor sensitivity

Spectral sensitivity of the three identified photoreceptor classes was modeled by multiplying their absorbance spectra by the transmittance spectra of elements in their light path. For UV and green cells, these were corneal lens and vitreous humor. Absorbance of green photoreceptors proximal to the red filter pigment was additionally multiplied by filter transmittance at a total filter depth of 28 μm .

Predicted effects on discriminability of male ornaments

We measured the reflectance of four discrete color patches displayed by *H. pyrrithrix* males to females during courtship: the green ventral surface of the first leg pair, the cream dorsal surface of the pedipalps, the red center of the face, and the anterior-facing orange patch on the third leg pair. These colors are produced by a combination of light scattering and pigmentary absorbance from the cuticular exoskeleton and modified hairs on its surface. We measured reflectance spectra from ca. 200 μm^2 areas of these body surfaces in 1 nm increments from 300-800 nm via MSP (20/20 PV™ Microspectrophotometer, CRAIC Technologies, San Dimas, CA) calibrated using a Spectralon® diffuse reflectance standard (Labsphere, Inc., North Sutton, NH). Color patches were illuminated via a focused xenon light source whose beam axis was oriented normal to the surface and coincident with the axis of light collection. To evaluate the potential value of trichromatic vision for females viewing courting males, we used a receptor-noise limited color opponency model (log-transformed quantum catch) of color vision [S8] to estimate differences in discriminability of male ornaments for a *H. pyrrithrix* eye with two photoreceptor classes

(dichromatic, UV and green only), versus an eye that includes a sub-population of green photoreceptors that have been shifted towards a longer wavelength peak (trichromatic, UV, green and 'red' photoreceptors). We estimated relative abundance of the three photoreceptor classes in the center of the tiered salticid retina using morphological information derived from the sectioning described above and published salticid retinal ultrastructure [S9-11]. Photoreceptor densities increase in more proximal tiers, with the highest photoreceptor densities found in tier I. Our vision models were constructed with an estimated photoreceptor ratio of 1 (UV in tiers III and IV) : 2 (green in tier II) : 4 (red in tier I) photoreceptors. Assuming a Weber fraction of 0.05, we compare perceptual distances (ΔS^i) of male ornament patch contrasts when viewed by a di- and trichromatic salticid eye. Because behaviorally determined color thresholds are not currently available for salticids, this metric provides an estimate of relative increase in discriminability.

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