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## Supplementary Materials

www.sciencemag.org/content/343/6169/408/suppl/DC1  
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# A Different Form of Color Vision in Mantis Shrimp

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One of the most complex eyes in the animal kingdom can be found in species of stomatopod crustaceans (mantis shrimp), some of which have 12 different photoreceptor types, each sampling a narrow set of wavelengths ranging from deep ultraviolet to far red (300 to 720 nanometers) (1–3). Functionally, this chromatic complexity has presented a mystery (3–5). Why use 12 color channels when three or four are sufficient for fine color discrimination? Behavioral wavelength discrimination tests ( $\Delta\lambda$  functions) in stomatopods revealed a surprisingly poor performance, ruling out color vision that makes use of the conventional color-opponent coding system (6–8). Instead, our experiments suggest that stomatopods use a previously unknown color vision system based on temporal signaling combined with scanning eye movements, enabling a type of color recognition rather than discrimination.

Stomatopods are benthic marine crustaceans that are generally found in tropical and temperate waters. Their compound eyes possess the largest number of photoreceptor types known in any animal [between 16 and 21 different receptors in some species (1, 3, 9)], allowing them to discriminate color (5) as well as both linear and circular polarized light (3, 10). Such retinal complexity is unrivaled in the animal kingdom, although papilionid butterflies may have up to eight spectral sensitivities (11). Theoretical approaches have predicted that between four and seven photoreceptor types are all that is needed to accurately encode the colors of the visible spectrum (12–14). The four-channel (tetrachromatic) solution that birds and reptiles use to sample a spectral range from 300 to 700 nm is optimally arranged to encode the known colors within this range. Where the spectrum examined loses the ultraviolet (UV) or red end, three photoreceptors

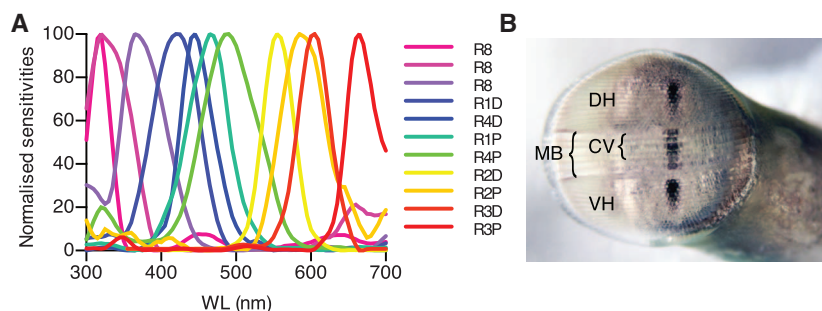
suffice, and trichromacy is the solution that many animals exhibit (12).

Our question was therefore, why do the stomatopods use 12 different photoreceptors to encode color? Before the experiments described here, Marshall *et al.* (5) demonstrated that stomatopods are capable of simple color discriminations based on color-card tests, similar to those devised by Carl von Frisch for bees and now used widely for

a number of animals (15). We hypothesized two alternative mechanisms for color information processing in stomatopods: (i) a multiple dichromatic color-opponent system (as described below), or (ii) the binning of colors into 12 separate channels, without any between-channel comparisons (4, 16).

Like butterflies, stomatopods have a variety of colorful body patterns, even using fluorescence to enhance color display (17). Furthermore, many of these species inhabit shallow coral reefs, one of the most colorful environments on Earth. The stomatopod's colors are thought to be involved in particularly complex communication systems, both between and within species (18), but little of this complexity requires a 12-dimensional color space to distinguish the colors available. Osorio *et al.* (19) speculated that stomatopods use their color sense to make reliable and quick judgements of color signals from conspecifics under changing light conditions, their steep-sided spectral sensitivities allowing particularly good color constancy. This would require comparison of the spectrally adjacent sharply tuned spectral sensitivities, and there is some anatomical evidence supporting this idea (6).

Stomatopod eyes are made up of a dorsal and ventral hemisphere, divided by a region of distinct



**Fig. 1. (A) Spectral sensitivities of *H. trispinosa*.** Spectral sensitivity curves obtained from intracellular electrophysiological recordings. The figure shows smoothed data (four neighbors on each side, second-order polynomial), normalized to 100% (see table S1). **(B) Eye of *H. trispinosa*.** Showing the dorsal hemisphere (DH) and ventral hemisphere (VH), divided by the midband (MB) containing the color receptors in the four top rows (CV).

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ommatidia (optical units) termed the midband (Fig. 1). Within two superfamilies of stomatopods (Gonodactyloidea and Lysiosquilloidea), this midband consists of six separate rows of ommatidia, each with different functionalities (2). Rows 1 to 4 are involved in color processing; rows 5 and 6 mediate the detection of circular or linear polarized light (3). A total of 12 cell types, each with different spectral sensitivities, are found within rows 1 to 4, with four UV-sensitive reticular cells located distally in the retina and by convention termed R8 cells (3). Beneath the R8 cells, the remaining seven reticular cells (R1 to R7) are further divided into two tiers (2). Comparison of the secondary R1 to R7 cell tiers would yield a set of highly tuned dichromatic mechanisms, sampling the 400- to 700-nm part of the spectrum in four bins as per hypothesis (i) above (3, 6, 19) (Fig. 1). Hypothesis (ii) would require a recognition of the pattern of excitation over the entire spectrum.

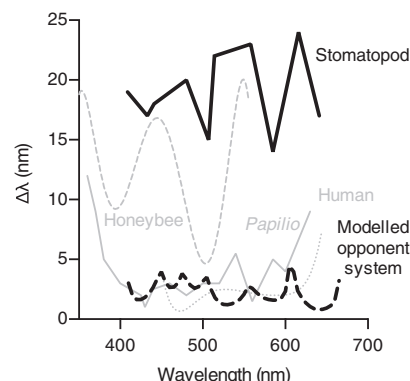
To distinguish between the two proposed hypotheses, we tested the ability of stomatopods to distinguish between different hues [spectral discrimination ( $\Delta\lambda$ ) functions] using the Gonodactyloid stomatopod species *Haptosquilla trispinosa*. When two narrow-band spectral stimuli are presented simultaneously, they can only be discriminated when the difference between them is over a certain threshold, giving the minimum discriminable difference. Spectral discrimination curves obtained from other animals usually exhibit certain minima

in areas between the spectral sensitivity curves (20). Therefore, dichromats usually exhibit one such minimum, trichromats have two minima, tetrachromats have three, and so on. Our goal was therefore to test the color discrimination abilities of mantis shrimp by using a two-way choice test (Fig. 2 and fig. S1) in which the animal is trained to a specific wavelength by means of food rewards. The wavelength stimuli were presented to the animal with a pair of optical fibers, and a choice was recorded when the animal grabbed or tapped the end of the fiber. Test colors were presented together with the trained colors at varying wavelength intervals to determine at what point the animal could no longer discriminate between the two stimuli (i.e., when the success rate dropped to 50%).

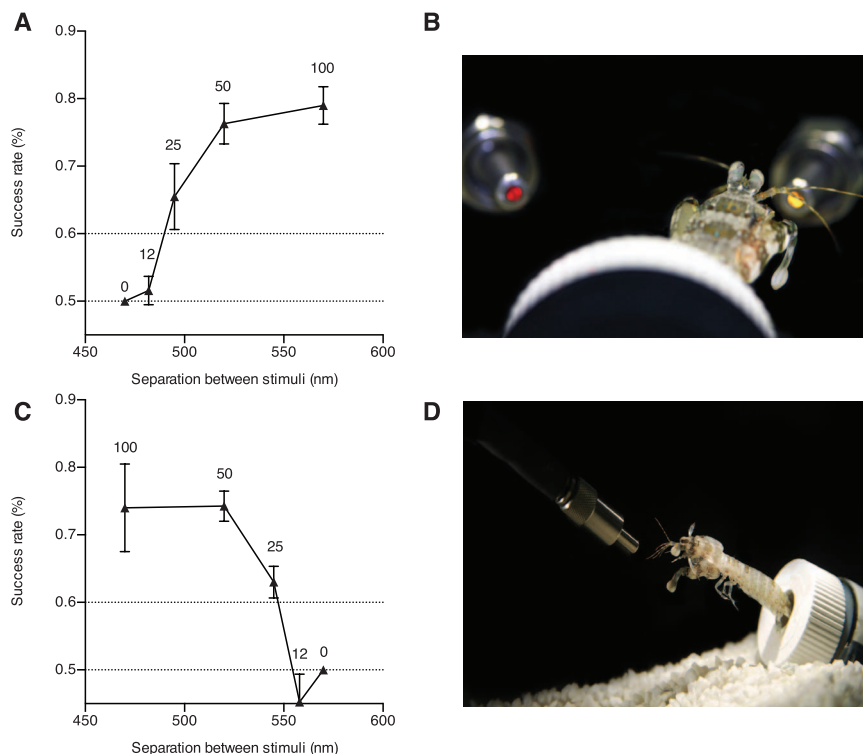
Animals were trained successfully to 10 different color wavelengths: 400, 425, 450, 470, 500, 525, 570, 578, 628, and 650 nm. When the test wavelength was 50 to 100 nm from the trained wavelength, the success rates were between 70 and 80%, indicating that they discriminated well between the two stimuli. However, when the interval between the trained and test wavelengths was reduced to between 25 and 12 nm, the success rates dropped to around 50%, and it was clear that the stomatopods could no longer distinguish test from trained stimuli. An example of success rates is given in Fig. 2, and further results are shown in tables S2 and S3. The discrimination threshold was chosen to be at a 60% success rate, in accordance

with previous studies on animal discrimination thresholds (20). Using the interpolated points at 60%, we determined a relative spectral discrimination curve of  $\Delta\lambda/\lambda$  (Fig. 3). The resulting values of  $\Delta\lambda$  were all in the region between 12 and 25 nm, and the prominent dips, or minima, usually associated with the points between spectral sensitivity maxima in other studies were not clearly defined by spectral overlap regions (Fig. 3).

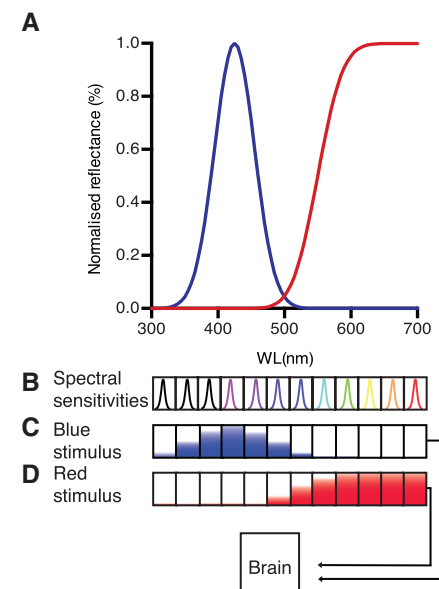
The potential spectral discrimination ability, based on previously known color vision systems, was also modeled to allow us to make predictions about the stomatopod color processing system. The



**Fig. 3. Spectral discrimination curves ( $\Delta\lambda/\lambda$ ).** The spectral discrimination curve from behavioral testing of *H. trispinosa* is shown by a thick black line, and the modeled spectral discrimination curve is shown by a thick dashed line. [The figure is modified from Koshitaka *et al.* (20).]



**Fig. 2. Examples of correct choice data from a two-way choice test of *H. trispinosa*.** Curves are plotted as mean  $\pm$  SEM. The horizontal dashed lines indicate the 50% (chance) and 60% (discrimination) criteria. (A) Choices of animals trained to 470 nm ( $n = 7$ ) and tested toward longer wavelengths. (C) Choices of animals trained to 570 nm ( $n = 4$ ) and tested toward shorter wavelengths. The number above each point indicates the tested wavelength interval. (B and D) Examples of animals making a choice.



**Fig. 4. Proposed processing mechanism.** (A) Idealized spectral reflectance from stomatopod body parts. WL, wavelength. (B) Spectral sensitivities throughout the spectrum divided into separate bins. (C and D) Excitation patterns of each spectral sensitivity when looking at the blue (C) and red (D) reflectance spectra.

sensitivities of the photoreceptors in *H. trispinosa* were measured using intracellular electrophysiology (9) (table S1). Eight sensitivity maxima were found in the visible part of the spectrum, and another three were found in the UV {the fourth UV cell often proving hard to locate and record from [(9), Fig. 1]}. We modeled the stomatopod spectral discrimination curve using the Vorobyev/Osorio noise-limited model (21) (which determines color thresholds using photoreceptor noise levels) for a serial dichromatic system with comparison between each adjacent spectral sensitivity [mechanism (i) above (note S1)]. This system predicts very fine discrimination between 1 to 5 nm throughout the spectrum, with few peaks of coarser discrimination as seen in other animals. Such fine spectral discrimination would be expected in a color vision system that made analog comparisons between adjacent spectral sensitivities. The behavioral results presented here (Fig. 2) suggest that such analog comparisons are not made. Instead, stomatopod color vision is remarkably coarse (Fig. 3).

The results from our experiments suggest that the stomatopods do not use a processing system of multiple dichromatic comparisons as previously hypothesized based on assumed neural connections (16). Instead, we provide evidence that scanning eye movements (22) may generate a temporal signal for each spectral sensitivity, enabling them to recognize colors instead of discriminating them. (Fig. 4) (3, 4). In such a system, the 12 sensitivities (including the UV, not analyzed here but with its multiple sensitivities a good fit to the system envisaged) would be converted into a temporal pattern when scanned across an object, which the animal could recognize as color. This system is comparable to the spectral linear analyzers (termed "push-broom" analyzers because of the arrangement of the sensors and the flight direction) used in remote sensing systems (23) and is a unique way for animals to encode color. Although this system does not have the ability to discriminate between closely positioned wavelengths (and results in spectral "discrimination" defined by the distances between sensitivity peaks, seen when comparing Fig. 1 and 3), it would enable the stomatopod to make quick and reliable determinations of color, without the processing delay required for a multidimensional color space. Without the comparison of spectral channels, color constancy would not function in the way we currently understand it in other animals. Instead, identification of a color pattern by the mid-band and luminance by the hemispheres might function as a "panchromatic" method to discount illuminance (23). The eye is optically skewed so that both midband and hemispheres examine the same areas in space, which lends support to this idea (3). However, the details of the neural processing from the receptors remain unknown.

Stomatopods live a rapid-fire lifestyle of combat and territoriality, so possessing a simple, temporally efficient color recognition ability may be critical for survival (24, 25). As with many invertebrate information-encoding solutions, the actual processing of the problem is dealt with at

the periphery, in this case by an array of detectors seen in animals and unconsciously duplicated by remote-sensing engineers. What remains for us to discover is the nature of the information and its importance in the biological decisions these enginging crustaceans make.

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#### Supplementary Materials

www.sciencemag.org/content/343/6169/411/suppl/DC1  
Materials and Methods  
Figs. S1 and S2  
Tables S1 to S3  
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## Risky Ripples Allow Bats and Frogs to Eavesdrop on a Multisensory Sexual Display

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Animal displays are often perceived by intended and unintended receivers in more than one sensory system. In addition, cues that are an incidental consequence of signal production can also be perceived by different receivers, even when the receivers use different sensory systems to perceive them. Here we show that the vocal responses of male túngara frogs (*Physalaemus pustulosus*) increase twofold when call-induced water ripples are added to the acoustic component of a rival's call. Hunting bats (*Trachops cirrhosus*) can echolocate this signal by-product and prefer to attack model frogs when ripples are added to the acoustic component of the call. This study illustrates how the perception of a signal by-product by intended and unintended receivers through different sensory systems generates both costs and benefits for the signaler.

**E**laborate courtship displays are favored by sexual selection but are often opposed by predation (1, 2). Animals can produce complex signals that are detected and processed through multiple sensory systems [commonly referred to as multimodal or multisensory signals (3, 4)]. Many communication systems once thought to operate primarily in a single sensory mode actually include secondary components that stimulate additional

senses (5–7). For instance, lip movements are necessary to produce human speech, but the associated visual cue of moving lips can also have a dramatic impact on speech perception (7). Such secondary signal components can be beneficial when they enhance the detection and perception of signals (3, 4). However, communication between the sender and intended receiver rarely occurs in private channels (8), and signalers are prone to costs imposed by eavesdroppers, such as predators and parasites who also attend to their displays (9–11).

Many male frogs possess conspicuous vocal sacs that evolved to recycle air during the production of their advertisement calls (6). The inflation and deflation of the vocal sac additionally act as

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