

# Spectral discrimination in color blind animals via chromatic aberration and pupil shape

Alexander L. Stubbs<sup>a,b,1,2</sup> and Christopher W. Stubbs<sup>c,d,2</sup>

<sup>a</sup>Museum of Vertebrate Zoology, University of California, Berkeley, CA 94720; <sup>b</sup>Department of Integrative Biology, University of California, Berkeley, CA 94720; <sup>c</sup>Department of Physics, Harvard University, Cambridge, MA 02138; and <sup>d</sup>Department of Astronomy, Harvard University, Cambridge, MA 02138

Edited by John Mollon, University of Cambridge, Cambridge, United Kingdom, and accepted by Editorial Board Member Jeremy Nathans May 23, 2016 (received for review December 13, 2015)

We present a mechanism by which organisms with only a single photoreceptor, which have a monochromatic view of the world, can achieve color discrimination. An off-axis pupil and the principle of chromatic aberration (where different wavelengths come to focus at different distances behind a lens) can combine to provide “color-blind” animals with a way to distinguish colors. As a specific example, we constructed a computer model of the visual system of cephalopods (octopus, squid, and cuttlefish) that have a single unfiltered photoreceptor type. We compute a quantitative image quality budget for this visual system and show how chromatic blurring dominates the visual acuity in these animals in shallow water. We quantitatively show, through numerical simulations, how chromatic aberration can be exploited to obtain spectral information, especially through non-axial pupils that are characteristic of coleoid cephalopods. We have also assessed the inherent ambiguity between range and color that is a consequence of the chromatic variation of best focus with wavelength. This proposed mechanism is consistent with the extensive suite of visual/behavioral and physiological data that has been obtained from cephalopod studies and offers a possible solution to the apparent paradox of vivid chromatic behaviors in color blind animals. Moreover, this proposed mechanism has potential applicability in organisms with limited photoreceptor complements, such as spiders and dolphins.

spectral discrimination | chromatic aberration | color vision | pupil shape | cephalopod

We show in this paper that, under certain conditions, organisms can determine the spectral composition of objects, even with a single photoreceptor type. Through computational modeling, we show a mechanism that provides spectral information using an important relationship: the position of sharpest focus depends on the spectral peak of detected photons. Mapping out contrast vs. focal setting (accommodation) amounts to obtaining a coarse spectrum of objects in the field of view, much as a digital camera attains best focus by maximizing contrast vs. focal length. We note that a similar phenomenon has been advanced as a possible explanation for color percepts in red/green color-blind primates (1); however, primates have not evolved the off-axis pupil shape found in nearly all shallow water cephalopods that enhances this effect.

The only other known mechanism of color discrimination in organisms involves determining the spectrum of electromagnetic radiation using differential comparisons between simultaneous neural signals arising from photoreceptor channels with differing spectral acceptances. Color vision using multiple classes of photoreceptors on a 2D retinal surface comes at a cost: reduced signal to noise ratio in low-light conditions and degraded angular resolution in each spectral channel. Thus, many lineages that are or were active in low-light conditions have lost spectral channels to increase sensitivity (2).

Octopus, squid, and cuttlefish (coleoid cephalopods) have long been known to be among the most colorfully active organisms, vividly changing color to signal conspecifics and camouflage. In 350 BCE, Aristotle (3) remarked that the octopus “seeks its prey by

so changing its color as to render it like the color of the stones adjacent to it; it does so also when alarmed.”

Cephalopods use their control of skin coloration to become (i) inconspicuous by camouflaging against local backgrounds (Fig. 1, Fig. S1, and Movie S1) or (ii) highly conspicuous during colorful mating and threat displays (Fig. 1, Fig. S2, and Movie S2). Despite this chromatically active behavior, genetic and physiological studies (4–7) show that (with one exception) cephalopods lack multiple photoreceptor types. Cephalopods also fail certain behavioral trials (7–11) designed to test for color vision by opponent spectral channels.

We are faced with two distinct but related paradoxes: (i) how can these animals with a single photoreceptor achieve good background color matching, and (ii) why would they break camouflage to produce risky colorful mating displays (readily visible to predators with color vision) unless this chromatic information was visible to conspecifics and carried some selective advantage?

Previous attempts to reconcile these apparent paradoxes include suggestions that (i) the animals do not actually match natural background colors (12) or (ii) multiple photoreceptor types could exist (13, 14) in the animal’s skin. Neither of these explanations resolves the puzzle of “color-blind camouflage,” and researchers remain in search of a mechanism that allows for this ability (7, 14–16). We are unaware of a proposal for how natural selection would drive the evolution and maintenance of colorful intraspecific displays in these soft-bodied mollusks if this information was not available to the animals themselves.

## Significance

We describe a means of obtaining spectral information using the principles of physical optics and an off-axis pupil shape without requiring spectrally distinct photoreceptor classes. The mechanism described here offers a possible solution to a long-standing puzzle in marine animals: cephalopods dramatically change color for both producing chromatically matched camouflage and signaling to conspecifics, despite having a single photoreceptor channel. The ability of these animals to achieve such excellent color matching to their surroundings, despite being “color blind” in the traditional sense, can be understood if they exploit chromatic aberration to deduce spectral information. The bizarre off-axis pupils of these animals can be understood as an adaptation that maximizes spectral information, even at the expense of image acuity.

Author contributions: A.L.S. and C.W.S. designed research, performed research, analyzed data, and wrote the paper.

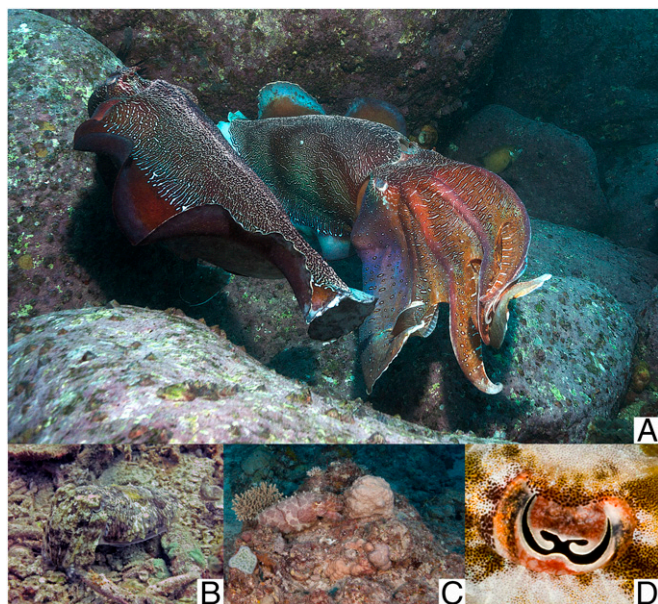
The authors declare no conflict of interest.

This article is a PNAS Direct Submission. J.M. is a guest editor invited by the Editorial Board. Freely available online through the PNAS open access option.

<sup>1</sup>To whom correspondence should be addressed. Email: astubbs@berkeley.edu.

<sup>2</sup>A.L.S. and C.W.S. contributed equally to this work.

This article contains supporting information online at [www.pnas.org/lookup/suppl/doi:10.1073/pnas.1524578113/-DCSupplemental](http://www.pnas.org/lookup/suppl/doi:10.1073/pnas.1524578113/-DCSupplemental).



**Fig. 1.** Cephalopod behavior and pupil shapes. [Figs. S1 and S2](#) and [Movies S1 and S2](#) show additional examples. Many shallow water cephalopods produce colorful displays [(A) Australian giant cuttlefish *Sepia apama*] to conspecifics and accurately color-match natural environments to camouflage [(B and C) broad-club cuttlefish *Sepia latimanus*]. Their pupil shapes [(D) *S. bandensis*] maximize chromatic blur. Images courtesy of (A) Klaus Stiefel, (B) Flickr/Lakshmi Sawitri, (C) Ken Marks, and (D) Roy Caldwell.

### Contradictory Evidence: Chromatic Behavior but a Single Opsin

The extent of color matching in cephalopods remains somewhat controversial in some circles, but we assert that shallow water cephalopods often match the coloration of natural backgrounds (Fig. 1, [Fig. S1](#), and [Movie S1](#)), and we encourage readers to examine [Movie S1](#), which shows cephalopod camouflage in their natural habitat, and reach their own conclusions. Some had claimed (12) that these organisms simply match the brightness and spatial scale of patterns in their environment, tricking the human visual system without actually requiring a color match. Numerous studies (14, 17–21) show, however, that cuttlefish and octopus actively vary their spectral reflectance in response to background color rather than simply modulating their luminance.

Kühn (21) conducted a series of behavioral experiments comparing the octopus and cuttlefish camouflage responses when placed on a series of greyscale and colored substrates. His data show statistically significant evidence that these organisms expand their long wavelength-reflecting chromatophores when on spatially variable red or yellow backgrounds but that they primarily expand black chromatophores when on corresponding greyscale backgrounds (21). Kühn (21) concluded that these organisms must have the ability to discriminate spectral content.

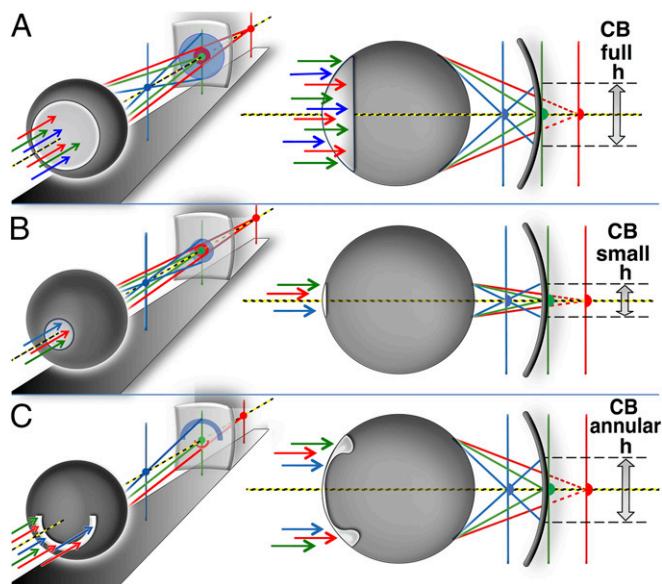
Contemporary laboratory and field observations (17–20) show that octopus and cuttlefish produce high-fidelity color matches to natural backgrounds (Fig. 1). The most definitive recent evidence for color matching in a laboratory setting used (14) a hyperspectral imager in conjunction with spectral angle mapping to show that cuttlefish varied their spectral reflectance (chromatic properties) to maintain excellent spectral matches to a diversity of natural backgrounds and interestingly, maintained poorer matches in brightness (luminance). These studies (14, 17–20) corroborate the earlier result by Kühn (21): cephalopods vary their spectral reflectance by active control over their chromatophores in response to natural backgrounds rather than simply varying their luminance.

Some have suggested that cephalopod skin might contribute to spectral discrimination through either undiscovered additional opsins (14) or filtering the single known opsin response. The recently published octopus genome (22) did not identify any additional opsins using both whole-genome sequencing and transcriptome sequencing of skin tissue, despite a focus on identifying G protein-coupled receptors. Across a diversity of taxa, all cephalopod studies to date have found rhodopsin transcripts in the skin identical to those in the eye (23), and the skin's spectral response to light is nearly identical to that of the retina (24). Given multiple strong lines of evidence against additional undiscovered skin opsins and no described mechanism for spectral discrimination arising from rhodopsin alone, this competing hypothesis is not currently viable. Additionally, absent a focusing element, detectors on the skin act as wide-angle nonimaging light sensors and cannot provide useful information regarding background coloration or signals produced by conspecifics.

### Chromatic Blurring and the Importance of Pupil Shape

Fig. 2 shows the mechanism that we are proposing for how chromatic aberration can be exploited to achieve spectral sensitivity. As we show below, the off-axis pupils of cephalopods combine with the wavelength dependence of the lens index of refraction to generate chromatic blur; different wavelengths come into focus at different distances from the lens. The spectral content of a structured scene can be deduced by sweeping through focus (i.e., changing the lens to retina distance) and seeing how the image blurring varies. A key element in our argument is the observation that the off-axis pupils common in cephalopods actually maximize the chromatic blurring in their visual system ([Table S1](#)). These animals would have better acuity if they had evolved a small, on-axis pupil, such as the one in the eye of the reader. Instead, they seem to have sacrificed overall acuity in favor of chromatic blurring, which we suggest here as a mechanism for spectral discrimination. This mechanism is similar to that in the recent observation (25) that vertical and horizontal pupils produce astigmatic blurring.

[SI Experimental Procedures](#) provides a detailed description of the numerical modeling that we performed to assess the



**Fig. 2.** Chromatic blur and pupil geometry. The (A) full and (C) annular aperture pupils produce more chromatic blurring (CB) than (B) the small on-axis pupil, because they transmit rays with a larger ray height  $h$ . Vertical lines show best focus positions for blue, green, and red light.

quantitative variation of blurring vs. spectral structure and focal spacing. These calculations were based on the measured optical properties of cephalopods, and we show that (despite claims to the contrary in previous works by others) chromatic blurring dominates the image quality for these animals. This chromatic aberration is what affords them the opportunity to exploit this mechanism for achieving color sensitivity.

## Results

Ideally, a set of monochromatic measurements of the point spread function produced by a cephalopod lens for different pupil sizes and lens to retina spacing would establish an empirical determination of the chromatic blur seen by these creatures. We are unaware of an appropriate comprehensive dataset, and therefore we have used the available laboratory measurements to produce a computer model of the chromatic properties for a representative cephalopod. Because the primary eye design features (complex pupil shape, spherical gradient index lens, and single-opsin retina) are common across coleoid cephalopods, we will use this model as representative of this class of animals.

Using measured (26) optical properties of *Octopus australis*, we performed a simulation by constructing a hyperspectral image cube [at 5  $\mu\text{m}/\text{pixel}$  in the spatial directions, corresponding to a typical cephalopod rhabdome diameter (12, 27), and 200 planes spanning 450 nm <  $\lambda$  < 650 nm in the spectral direction at  $\Delta\lambda = 1$  nm]. We modeled an  $f/1.2$  spherical lens with a 10-mm diameter, but our computed chromatic blurring results are independent of this choice of length scale. For each lens to retina focal distance, which brings a single wavelength into crisp focus, we computed the pupil-dependent chromatic image blur at the other wavelengths. We summed up the blurred image cube along the wavelength direction [weighted by the product of the seawater-filtered solar photon illumination, the reflectance spectrum, and the opsin response curve (Fig. S3)] to arrive at a final simulated chromatically blurred image on the retina. This procedure was repeated for three different pupil shapes for a sequence of accommodation values.

**Chromatic Blur Dominates Image Quality Budget.** A variety of factors determine the blurring of the image formed on the retina, including diffraction through the pupil, aberrations in the optical system, and retinal limitations (2, 25–34). These terms comprise the image quality budget and determine the sharpest image that can be formed. The eyes of *O. australis* are particularly well-studied (26), and we used data from this species as a proxy for other shallow water cephalopod eyes to make quantitative assessments of the image quality budget. The *O. australis* lenses have two properties shared by all other studied cephalopods: (i) they are remarkably well-corrected for spherical aberration, and (ii) the index of refraction varies with wavelength, inducing chromatic aberration. This chromatic aberration is uncorrected and found in all studied (26, 28, 29) cephalopod lenses. In some other animals, radial multifocal zones may produce a partial chromatic correction (30).

Wavelength dependence of the index of refraction induces (26) chromatic blurring, because different wavelengths have different focal lengths. This effect dominates the image quality budget (Table S1). The extent of chromatic blurring depends on both chromatic focal shift and the angle at which rays strike the optical axis (Fig. 2). This angle depends on the ray's height  $h$ ; the off-axis ray distribution determines the extent of chromatic blur. Although the single opsin restricts the range of wavelengths detected, our analysis shows that, when integrated over the wavelength response, chromatic blurring dominates image quality, except for small, on-axis pupils or when the lens diameter is so small that the granularity of the photoreceptors dominates. A monochromatic point source generates a scaled image of the pupil, with both size and parity determined by the amount of defocus (Fig. 2 and Fig. S4 A–C).

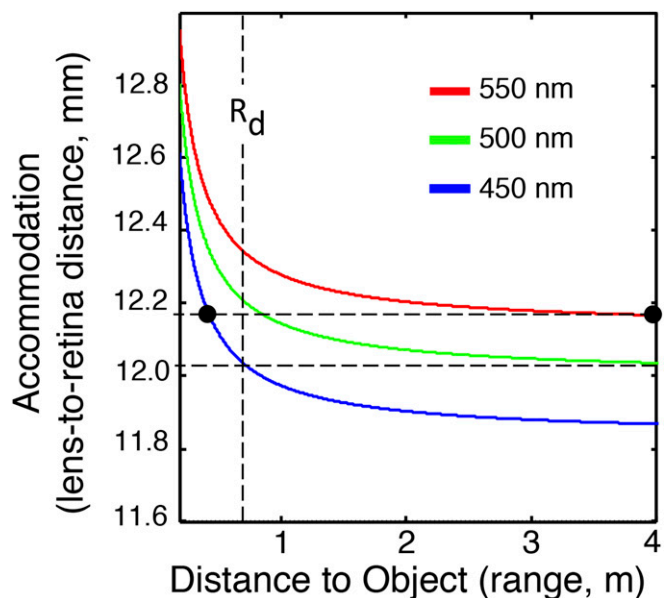
**Range, Color, and Best Focus.** Organisms routinely determine the best focus for objects of interest in their visual field by varying focal length and comparing relative image quality. This focus aberration can be used as an accurate range-finding mechanism as shown in chameleons (35) and jumping spiders (36). If chromatic blur dominates the image quality budget, there is an interrelationship between range, color, and best focus (Fig. 3). For example, jumping spiders misjudge (36) distance depending on the illumination spectrum. Differential image blurring has been proposed (37) as a range-finding mechanism for squid, but chromatic aberration [not considered in the work by Chung and Marshall (37)] drives a strong relationship between spectrum, range, and best focus. Even in this narrowband system, chromatic aberration can compromise the determination of range based on best focus values.

Coleoid cephalopods use (38) binocular convergence and stereopsis to judge distance when striking prey items with their projectile tentacles, independent of image acuity. Thus, the combination of a determination of best focus and an independent determination of range allows for spectral discrimination.

The spherical lens system that we modeled obeys a modified lensmaker's equation. The image distance  $I$  is a function of the wavelength-dependent focal length  $f(\lambda)$  and object distance  $O$ , with

$$\frac{1}{I} = \left[ \frac{1}{\cos(\text{asin}(\frac{R}{O}))} \right] \left[ \left( \frac{1}{f(\lambda)} \right) - \left( \frac{1}{O} \right) \right].$$

We used this expression and the measured (26) chromatic aberration for *O. australis* to compute the image distance  $I$  needed to achieve a focused image as a function of both object distance and wavelengths at  $\lambda = 450, 500,$  and  $550$  nm. These wavelengths correspond to the opsin peak and the FWHM of



**Fig. 3.** Range–color–focus relationship for a 10-mm-diameter cephalopod lens. Colored lines show the accommodation vs. range relationship at the 500-nm opsin peak sensitivity and the FWHM of the spectral resolution that we estimate for the chromatic focusing hypothesis. For objects more distant than  $R_d$ , there is an unambiguous relationship between wavelength and the accommodation setting that makes the sharpest image. For objects closer than  $R_d$ , an independent determination of range is needed to break the degeneracy between range and color.

the spectral resolution that we estimated numerically using synthetic monochromatic illumination. Fig. 3 shows the relationship between object distance (range) and the lens to retina spacing (accommodation) for these chosen wavelengths. Although there is color-range ambiguity for nearby objects, range-independent spectral discrimination (defined here as discrimination between the opsin peak wavelength and the two FWHM points) can be achieved for objects at distances beyond  $R_d = (d/(10 \text{ mm}))(0.75 \text{ m})$  for lens diameter  $d$ . In this geometrical optics regime, the disambiguation range  $R_d$  scales with lens diameter.  $R_d$  is about one body length in *O. australis*.

Beyond  $R_d$ , best focus depends only on spectrum and is independent of range. A scan through focus amounts to a spectral scan of the scene. The animal can determine the object's color by finding the focal setting that produces the sharpest image, regardless of range. This best focus determination can be achieved by some combination of (i) displacing the lens (39) relative to the retina (accommodation), (ii) changing the distance to the object, and/or (iii) imaging the object across regions of the retinal surface with different effective focal lengths.

What occurs when objects are closer than  $R_d$ ? The focal spacing creating a crisp focus of a 450-nm light source at 0.2 m also creates a sharp image of a 550-nm light source more than 4 m away (Fig. 3). Studies of range determination in cuttlefish show (38) that they use multiple methods for precisely establishing distances. Both cuttlefish and squid rely on this ability to accurately project their tentacles and capture food. This ranging ability can break the range color degeneracy, improving spectral resolution and allowing them to use image sharpness to obtain spectral information for  $R < R_d$ .

This mechanism for spectral discrimination is computationally more intensive than a differential comparison of photoreceptor outputs in opponency. We believe that this may be one factor contributing to the exceptionally large (12) optic lobes found in coleoid cephalopods.

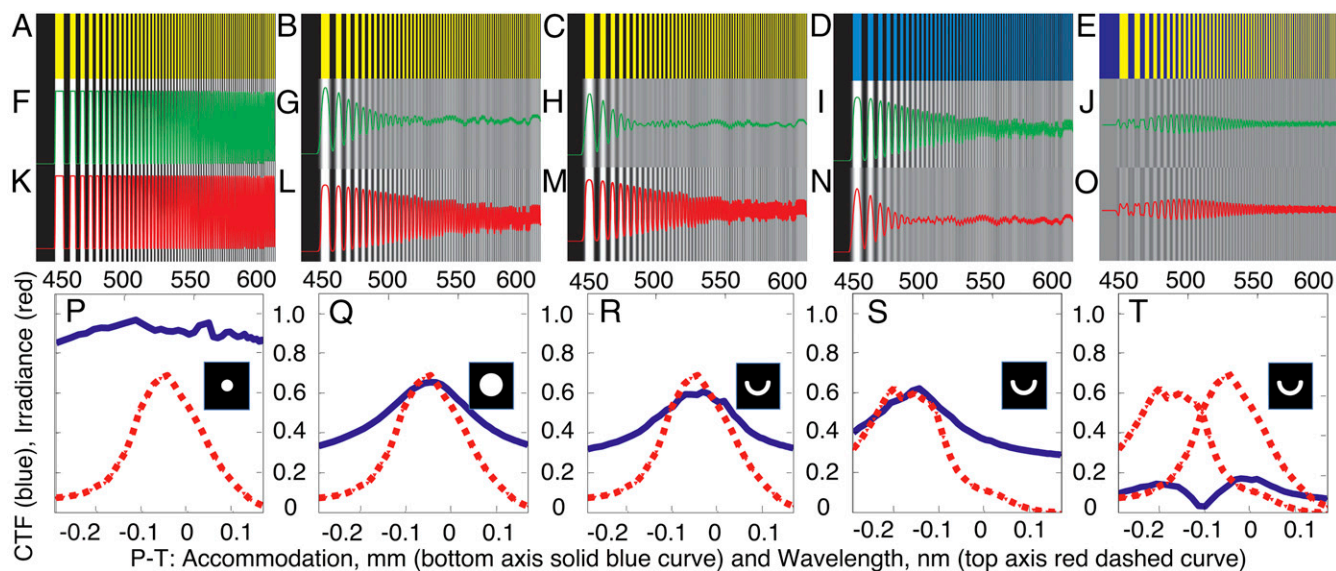
**Chromatic Blurring Evidently Favored over Visual Acuity.** Although ambient light levels influence optimal pupil area, pupil shape determines the extent of chromatic blurring (Fig. 2). Chromatic blur dominates the cephalopod image quality in low-light conditions with a fully dilated pupil (Table S1). The off-axis slit and semiannular pupils used in high-light conditions preserve this spectral discrimination mechanism across a wide dynamic range of illumination. The semiannular pupil shape (Fig. 1), common in both cuttlefish and shallow water squids, maximizes the off-axis distance of optical rays from objects in the horizontal plane around the animal. The horizontal slit pupil of shallow water octopus (Fig. S1F) species intercepts a similar ray bundle when imaging the bottom, acting as an arc-like pupil for images formed on the upper portion of the retina that has an enhanced density of photoreceptors (40).

We computed the pupil dependence of the contrast transfer function (CTF) vs. accommodation and the corresponding spectral resolution for three pupil shapes using the yellow to black test pattern (Fig. 4A–C). The small on-axis pupil (Fig. 4P) has minimal chromatic blur and maintains a crisp CTF across the range of accommodation settings, maximizing visual acuity but with degraded spectral sensitivity. Full (Fig. 4Q) and semiannular pupils (Fig. 4R) more realistically represent cephalopod pupils under low- and high-light conditions, respectively, and have virtually identical accommodation-dependent chromatic blur and correspondingly higher spectral resolution than the small pupil.

We propose that natural selection might favor the maintenance of spectral discrimination over image acuity in these animals.

## Discussion

Despite earlier behavioral results indicating color discrimination (9, 21), two lines of evidence drove (7) the prevailing view that nearly all cephalopods are color blind. First, only one photoreceptor type exists (4–6) in the retina of shallow water cephalopods. Our mechanism for spectral discrimination requires only one receptor type. Second, some behavioral experiments (7–11) designed to test for color vision in cephalopods produced negative results by using standard tests of color vision to evaluate the animal's ability to distinguish between



**Fig. 4.** Chromatic blur simulations for semiannular pupil. Test patterns (A–C in black and yellow, D in black and blue, and E in blue and yellow) are used to simulate chromatic blur vs. accommodation. Examples are shown of detected intensity variations and contrast at best focused wavelengths of (F–J) 470 and (K–O) 550 nm. CTF is extracted from line plots of intensity (traces in green in F–J and red in K–O). (P–T) CTF (blue) vs. accommodation (lower x scale) tracks the spectrum of detected photons (red) vs. wavelength (upper x scale), with a spectral resolution that depends on pupil shape. The pupil dependence of spectral resolution (width of blue CTF traces) is shown for (P) small ( $d = 1 \text{ mm}$ ) and (Q) full ( $d = 8 \text{ mm}$ ) on-axis pupils and (R–T) the semiannular ( $6 \text{ mm} < d < 6.66 \text{ mm}$ ) pupil. (R–T) The CTF peak tracks the spectrum for the semiannular pupil. (T) The flat CTF vs. accommodation obtained from (J and O) the line plots of intensity for (E) the blue to yellow test pattern precludes spectral discrimination for this case.

two or more adjacent colors of equal brightness. This adjacent color comparison is an inappropriate test for our model (Fig. 4R). Tests using rapidly vibrating (8, 9) color cues are also inappropriate. Although these dynamical experiments are effective tests for conventional color vision, they would fail to detect spectral discrimination under our model, because it is difficult to measure differential contrast on vibrating objects. These results corroborate the morphological and genetic evidence: any ability in these organisms for spectral discrimination is not enabled by spectrally diverse photoreceptor types.

Table S2 reviews cephalopod behavioral experiments that investigated color vision and their consistency with our proposed mechanism. Before the determination that cephalopods possess a single photoreceptor type, there were numerous experiments showing that they had spectral discrimination. These results were summarized and dismissed in the work by Messenger et al. (9) with the following rationale:

...all the authors are guilty of one or more of three serious errors: failure to take into account the spectral sensitivity curve of the subject, failure to control for the difference in brightness between test objects, and, in the behavioral experiments, inadequate quantification of results, which are presented without conventional statistical analysis.

We view this critique (9) of the 1950 paper by Kühn (21) as inaccurate. The work by Kühn (21) is mischaracterized as purely a training experiment (9). A reading of the work by Kühn (21) shows that, although he did perform extensive training experiments indicating spectral discrimination in *Octopus vulgaris*, he also clearly showed the differential responses of cuttlefish chromatophores to differentially colored textured backgrounds. We have provided a translation of the relevant section of this paper in *SI Experimental Procedures*.

In our proposed mechanism, cephalopods cannot gain spectral information from a flat-field background or an edge between two abutting colors of comparable intensity (Fig. 3). This phenomenology would explain why optomotor assays and camouflage experiments using abutting colored substrates (7, 9, 11) fail to elicit a response different from a flat-field background. Similarly, experiments (10) with monochromatic light projected onto a large uniform reflector or training experiments (8, 9) with rapidly vibrating colored cues would defeat a determination of chromatic defocus.

The mechanism proposed here is readily testable by conducting behavioral experiments that assess a cephalopod's ability to achieve successful camouflage as a function of both the spatial and the spectral structures of the background. Although we assert that the 1950 experiment by Kühn (21) clearly shows that the cuttlefish camouflage response differs in textured backgrounds colored in shades of gray with spectrally uniform reflectance compared with colored backgrounds, we suggest repeating this classic experiment using flat-field background of uniform luminosity without any potential focusing aids for the organisms.

We predict that the animals will fail to match flat-field backgrounds with no spatial structure as previously shown in figure 3B in the work by Mähger et al. (7) just as a photographer could not determine best focus when imaging a screen with no fine-scale spatial structure. If, for instance, their ability to spectrally match backgrounds was conferred by the skin or another potential unknown mechanism, they would successfully match on flat-field backgrounds. However, under our model, they should succeed when there is a spatial structure allowing for the calculation of chromatically induced defocus, such as in our test patterns (Fig. 4) or the more naturally textured backgrounds by Kühn (21). If, however, cephalopods truly cannot accurately match their background color but solely use luminance and achromatic contrast to determine camouflage, we would expect the response on colored substrates to be identical to that on a gray substrate of similar apparent brightness with identical spatial structure. We encourage groups with access to live

cephalopods to conduct these experiments, although we caution that they should be conducted under natural illumination conditions.

Our proposed mechanism has potential applicability in other species with a limited number of photoreceptor types and low  $f$ -number visual systems. Some dolphin species use (41) an annular pupil and a similar (42) radial gradient index of refraction lens uncorrected for chromatic aberration. They display evidence for behavioral color discrimination (43) in spectral regimes where their visual system would have difficulty (44) encoding color by opponent channels. More generally, a large number of organisms that are active both diurnally and nocturnally possesses (45) an annular pupil, and we wonder if these organisms could also benefit from color discrimination by our proposed mechanism.

Spider primary eyes also use a low  $f$ -number optical system and thus, induce high chromatic blurring. Additionally, most studied spiders image their environment with only two functional opsins (UV and green peak sensitivities) (36), although a recent study (46) showed that one genus of jumping spider may use retinal filtering to obtain some spectral discrimination. Some spiders also use an imaging system that maintains off-axis rays in high-light conditions (as in the cephalopod annular pupil) to simultaneously (47) image across multiple axially displaced focal planes. Jumping spiders can use image defocus across these focal planes to judge distance, but (as in cephalopods), this mechanism can be confounded (36) by color-range ambiguity (Fig. 3). When under natural sunlight, some jumping spiders exhibited a preference for red-colored mates (48), and crab spiders showed an ability to background match (49). However, these behaviors disappeared under fluorescent lighting (48, 49). Fluorescent lighting in these experiments created a series of line emissions that approximate  $\delta$ -functions and dominated the reflected spectrum from objects in the visual field. This illumination spectrum would make spectral inferences by chromatic defocus imaging difficult.

By simultaneously comparing image quality across multiple offset focal planes, these organisms might be able to obtain more spectral information than by photoreceptors working in opponency, and indeed, tiered retinas found (2) in spiders and many deep sea fish might represent the optimal morphology for spectral discrimination using our proposed mechanism.

## Conclusions

We have shown that the combination of off-axis pupil shape and chromatic aberration can be exploited to yield spectral information, albeit only in scenes that have substantial spatial/spectral contrast so that changes in chromatic blurring can be detected. A quantitative numerical model of the *O. australis* visual system shows the viability of this phenomenology. This spectral sensitivity mechanism offers a potential explanation for the apparent contradictions in cephalopod behavior in the wild, where these "color-blind" animals achieve remarkable color-matched camouflage and display in vivid colors. It is also consistent with the accumulated data from over 60 y of controlled laboratory vision experiments. This scenario may force us to rethink what it means to be a color-blind animal.

## Experimental Procedures

We computed the relationship between image sharpness, accommodation, and spectral content. We created test patterns with different spectral characteristics and simulated the images that they would form on the single-opsin retina of *O. australis* for different accommodation values. The test patterns (Fig. 4 A–E) are generated with the reflectance spectra (50) of blue and yellow Australian reef fish. The side length of each pixel in the test patterns is equal to the 5- $\mu$ m rhabdome diameter; our test images incorporate the sampling granularity inherent in this detector system.

We computed a CTF (Fig. 4) metric to map out image contrast as a function of accommodation, pupil shape, and the spectral content of the test image. The CTF vs. accommodation for a given test pattern tracks the underlying spectrum but with a spectral resolution that depends on intensity contrast as well as pupil shape. Fig. 4 P–T shows the spectral content of the test patterns (red lines) and the computed image sharpness vs. accommodation (blue lines). Table S1 shows

quantitative values of image blurring, and *SI Experimental Procedures* discusses how these were computed.

**Movie S3** is an animation that shows how the contrast of the image depends on focal setting (i.e., accommodation). Maximum contrast is obtained when the lens brings light at the peak of the detected photon spectrum into best focus. Two representative simulated blurred images are shown in Fig. 4 for each of five test patterns for focal settings that bring 470- and 550-nm light into focus on the retina. The amount of chromatic blurring is evident from their corresponding intensity line cuts shown as blue superimposed lines in the respective figures. We mapped image sharpness vs. accommodation setting over the full range of wavelengths. The spectral peak of the light detected from an object can be inferred from the accommodation setting where the image is best focused on the retina.

Under our model, the determination of spectral information is reliant on fine-scale intensity variations (edges, shadows, texture, etc.). This dependence imposes limitations. Cephalopods would be unable to determine the spectral content of a flat field of uniform color. They would similarly be unable to determine spectral information from abutting regions of comparable apparent

intensity, differing only in spectral content (Fig. 4 *E, J, O*, and *T*). This degeneracy can account for contradictory results obtained in laboratory behavior tests for color vision (Table S2).

Natural environments rich in shadows and structure serve as focusing aids. Spectra measured (50) in marine environments often provide the spectral structure needed for this mechanism. Intraspecific displays of these organisms (Fig. 1, Fig. S2, and Movie S2) typically exhibit adjacent fine-scale black and colored regions, facilitating best focus determination. We believe that this is another adaptation that favors our model.

**ACKNOWLEDGMENTS.** A.L.S. thanks University of California, Berkeley and the Museum of Vertebrate Zoology for support; Prof. J. McGuire for extensive opportunities, mentoring, and comments on this manuscript; and S. Johnsen, M. Banks, and R. Caldwell for helpful comments. C.W.S. acknowledges the support of Harvard University. We thank N.O.S.B. and the Packard Foundation for their support of science, J. Schoenberger for translation, and C. Gregory for editorial support. The MATLAB routines used for computing the results shown here will be made available on request.

- Forte JD, Blessing EM, Buzás P, Martin PR (2006) Contribution of chromatic aberrations to color signals in the primate visual system. *J Vis* 6(2):97–105.
- Cronin TW, Johnsen S, Marshall NJ, Warrant EJ (2014) *Visual Ecology* (Princeton Univ Press, Princeton).
- Aristotle (350 BCE) *A History of Animals*, trans Thompson DW (1910) (Clarendon, Oxford).
- Brown PK, Brown PS (1958) Visual pigments of the octopus and cuttlefish. *Nature* 182(4645):1288–1290.
- Bellingham J, Morris AG, Hunt DM (1998) The rhodopsin gene of the cuttlefish *Sepia officinalis*: Sequence and spectral tuning. *J Exp Biol* 201(Pt 15):2299–2306.
- Chung WS (2014) Comparisons of visual capabilities in modern Cephalopods from shallow water to deep sea. PhD dissertation (Queensland Brain Institute, University of Queensland, St. Lucia, QLD, Australia).
- Mähthger LM, Barbosa A, Miner S, Hanlon RT (2006) Color blindness and contrast perception in cuttlefish (*Sepia officinalis*) determined by a visual sensorimotor assay. *Vision Res* 46(11):1746–1753.
- Messenger JB (1977) Evidence that *Octopus* is colour blind. *J Exp Biol* 70(1):49–55.
- Messenger JB, Wilson AP, Hedge A (1973) Some evidence for colour-blindness in *Octopus*. *J Exp Biol* 59(1):77–94.
- Roffe T (1975) Spectral perception in *Octopus*: A behavioral study. *Vision Res* 15(3):353–356.
- Marshall NJ, Messenger JB (1996) Colour-blind camouflage. *Nature* 382(6590):408–409.
- Hanlon RT, Messenger JB (1998) *Cephalopod Behavior* (Cambridge Univ Press, Cambridge, United Kingdom).
- Mähthger LM, Roberts SB, Hanlon RT (2010) Evidence for distributed light sensing in the skin of cuttlefish, *Sepia officinalis*. *Biol Lett* 6(5):600–603.
- Chiao CC, Wickiser JK, Allen JJ, Genter B, Hanlon RT (2011) Hyperspectral imaging of cuttlefish camouflage indicates good color match in the eyes of fish predators. *Proc Natl Acad Sci USA* 108(22):9148–9153.
- Hanlon R (2007) Cephalopod dynamic camouflage. *Curr Biol* 17(11):R400–R404.
- Chiao CC, Chubb C, Hanlon RT (2015) A review of visual perception mechanisms that regulate rapid adaptive camouflage in cuttlefish. *J Comp Physiol A Neuroethol Sens Neural Behav Physiol* 201(9):933–945.
- Buresch KC, et al. (2015) Cuttlefish adjust body pattern intensity with respect to substrate intensity to aid camouflage, but do not camouflage in extremely low light. *J Exp Mar Biol Ecol* 462:121–126.
- Akkaynak D, Allen JJ, Mähthger LM, Chiao CC, Hanlon RT (2013) Quantification of cuttlefish (*Sepia officinalis*) camouflage: A study of color and luminance using in situ spectrometry. *J Comp Physiol A Neuroethol Sens Neural Behav Physiol* 199(3):211–225.
- Mähthger LM, Chiao CC, Barbosa A, Hanlon RT (2008) Color matching on natural substrates in cuttlefish, *Sepia officinalis*. *J Comp Physiol A Neuroethol Sens Neural Behav Physiol* 194(6):577–585.
- Hanlon RT, Chiao CC, Mähthger LM, Marshall NJ (2013) A fish-eye view of cuttlefish camouflage using in situ spectrometry. *Biol J Linn Soc Lond* 109(3):535–551.
- Kuhn A (1950) [Color change and color sense in cephalopods]. *Z Vgl Physiol* 32(6):573–598.
- Albertin CB, et al. (2015) The octopus genome and the evolution of cephalopod neural and morphological novelties. *Nature* 524(7564):220–224.
- Kingston ACN, Wardill TJ, Hanlon RT, Cronin TW (2015) An unexpected diversity of photoreceptor classes in the Longfin squid, *Doryteuthis pealeii*. *PLoS One* 10(9):e0135381.
- Ramirez MD, Oakley TH (2015) Eye-independent, light-activated chromatophore expansion (LACE) and expression of phototransduction genes in the skin of *Octopus bimaculoides*. *J Exp Biol* 218(Pt 10):1513–1520.
- Banks MS, Sprague WW, Schmoll J, Parnell JAQ, Love GD (2015) Why do animal eyes have pupils of different shapes? *Sci Adv* 1(7):e1500391.
- Jagger WS, Sands PJ (1999) A wide-angle gradient index optical model of the crystalline lens and eye of the octopus. *Vision Res* 39(17):2841–2852.
- Yamamoto T, Tasaki K, Sugawara Y, Tonosaki A (1965) Fine structure of the *Octopus* retina. *J Cell Biol* 25(2):345–359.
- Heidermanns C (1928) Messende Untersuchungen über das Formensehen der Cephalopoden und ihre optische Orientierung im Raume. *Zool Jahrb Abt Anat Ontogenie Tiere* 45:609–650.
- Sivak JG (1991) Shape and focal properties of the cephalopod ocular lens. *Can J Zool* 69(10):2501–2506.
- Kröger RHH, Campbell MCW, Fernald RD, Wagner HJ (1999) Multifocal lenses compensate for chromatic defocus in vertebrate eyes. *J Comp Physiol A Neuroethol Sens Neural Behav Physiol* 184(4):361–369.
- Gagnon YL, Sutton TT, Johnsen S (2013) Visual acuity in pelagic fishes and mollusks. *Vision Res* 92:1–9.
- Johnsen S (2012) *The Optics of Life: A Biologist's Guide to Light in Nature* (Princeton Univ Press, Princeton).
- Land MF, Nilsson DE (2012) *Animal Eyes* (Oxford Univ Press, Oxford).
- Jagger WS, Sands PJ (1996) A wide-angle gradient index optical model of the crystalline lens and eye of the rainbow trout. *Vision Res* 36(17):2623–2639.
- Harkness L (1977) Chameleons use accommodation cues to judge distance. *Nature* 267(5609):346–349.
- Nagata T, et al. (2012) Depth perception from image defocus in a jumping spider. *Science* 335(6067):469–471.
- Chung WS, Marshall J (2014) Range-finding in squid using retinal deformation and image blur. *Curr Biol* 24(2):R64–R65.
- Cheung Y, Mullins O, Nguyen P, Huberman A (2014) Extreme binocular plasticity and dynamic strategy implementation supports vision-dependent prey capture in cuttlefish. *Proceedings of the SACNAS National Conference*, ed G Miranda-Carboni (Society for the Advancement of Hispanics/Chicanos and Native Americans in Science, Santa Cruz, CA), p 1159.
- Schaeffel F, Murphy CJ, Howland HC (1999) Accommodation in the cuttlefish (*Sepia officinalis*). *J Exp Biol* 202(Pt 22):3127–3134.
- Talbot CM, Marshall JN (2011) The retinal topography of three species of coleoid cephalopod: Significance for perception of polarized light. *Philos Trans R Soc Lond B Biol Sci* 366(1565):724–733.
- Herman LM, Peacock MF, Yunker MP, Madsen CJ (1975) Bottle-nosed dolphin: Double-slit pupil yields equivalent aerial and underwater diurnal acuity. *Science* 189(4203):650–652.
- Kröger RHH, Kirschfeld K (1993) Optics of the harbor porpoise eye in water. *J Opt Soc Am A* 10(7):1481–1489.
- Griebel U, Schmid A (2002) Spectral sensitivity and color vision in the bottlenose dolphin (*Tursiops truncatus*). *Mar Freshwat Behav Physiol* 35(3):129–137.
- Fasick JJ, Cronin TW, Hunt DM, Robinson PR (1998) The visual pigments of the bottlenose dolphin (*Tursiops truncatus*). *Vis Neurosci* 15(4):643–651.
- Murphy CJ, Howland HC (1990) The functional significance of crescent-shaped pupils and multiple pupillary apertures. *J Exp Zool* 256(55):22–28.
- Zurek DB, et al. (2015) Spectral filtering enables trichromatic vision in colorful jumping spiders. *Curr Biol* 25(10):R403–R404.
- Blest AD, Hardie RC, McIntyre P, Williams DS (1981) The spectral sensitivities of identified receptors and the function of retinal tiering in the principal eyes of a jumping spider. *J Comp Physiol* 145(2):227–239.
- Taylor LA, McGraw KJ (2013) Male ornamental coloration improves courtship success in a jumping spider, but only in the sun. *Behav Ecol* 24(4):955–967.
- Llandres AL, Figon F, Christidès JP, Mandon N, Casas J (2013) Environmental and hormonal factors controlling reversible colour change in crab spiders. *J Exp Biol* 216(Pt 20):3886–3895.
- Marshall NJ, Jennings KA, McFarland WN, Loew ER, Losey GS (2003) Visual biology of Hawaiian reef fishes. III. Environmental light and an integrated approach to the ecology of reef fish vision. *Copeia* 2003(3):467–480.
- Morel A, Maritonrena S (2001) Bio-optical properties of oceanic waters: A reappraisal. *J Geophys Res* 106(C4):7163–7180.
- Schroeder DJ (1999) *Astronomical Optics* (Academic, San Diego).
- Groeger G, Cotton PA, Williamson R (2005) Ontogenetic changes in the visual acuity of *Sepia officinalis* measured using the optomotor response. *Can J Zool* 83(2):274–279.
- Muntz WRA, Gwyther J (1988) Visual acuity in *Octopus pallidus* and *Octopus australis*. *J Exp Biol* 134(1):119–129.
- Hao ZL, Zhang XM, Kudo H, Kaeriyama M (2010) Development of the retina in the cuttlefish *Sepia esculenta*. *J Shellfish Res* 29(2):463–470.
- Mahajan VN (1981) Zernike annular polynomials for imaging systems with annular pupils. *J Opt Soc Am* 71(1):75–85.