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## Color vision of the budgerigar (*Melopsittacus undulatus*): hue matches, tetrachromacy, and intensity discrimination

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**Abstract** Budgerigars, *Melopsittacus undulatus*, were trained to discriminate monochromatic lights from mixtures of two comparison lights. The addition of small amounts of UV (365 nm) to blue or yellow lights dramatically changed the color for the birds. Hue matches showed the birds to be dichromatic both at long wavelengths (only P565 and P508 active) and at short wavelengths (only P370 and P445 active because of screening of P508 and P565 by cone oil droplets). In mid-spectrum (only P445 and P508 active), a hue match was achieved, but the results were more complicated because two opponent neural processes were activated. All observed hue matches were in quantitative agreement with calculations of relative quantum catch in the pairs of participating single cones and point to the presence of a minimum of three opponent neural processes. For the hue matches at mid- and short wavelengths, the calculations also predict peak values of absorbance of the cone oil droplets associated with P508 and P445. Relative intensity of the training light affected difficult matches at long but not short wavelengths, likely due to achromatic signals from the double cones. With suitable training, birds could make intensity discriminations at short wavelengths, where the double cones have diminished sensitivity.

**Keywords** Budgerigar *Melopsittacus undulatus* · Color vision of birds · Hue matches · Ultraviolet vision · Intensity discrimination

### Introduction

The retinas of birds characteristically contain four spectral classes of single cones: long wavelength-sensitive cones (LWS) with peak absorption at 543–571 nm,

midwavelength-sensitive cones (MWS, 497–509 nm), short wavelength-sensitive cones (SWS, 430–463 nm), and an ultraviolet or violet-sensitive cone (UVS/VS) maximally sensitive at still shorter wavelengths. This latter type accounts for the most important source of variation among species studied to date. The presumptive ancestral form of this pigment (present, for example, in pigeons) has  $\lambda_{\max}$  at 402–426 nm, whereas a variety of passerines and the budgerigar have a UV-sensitive cone pigment differing by one or a few amino acid substitutions and with  $\lambda_{\max}$  shifted to 363–373 nm (Yokoyama et al. 2000; Ödeen and Håstad 2003; Hart 2004; reviewed in Cuthill 2005). Birds also possess double cones, both members of which have the LWS pigment and which account for somewhat more than half the cones in the retina. Unlike the cones of placental mammals, the inner segments of avian cone cells possess oil droplets containing high concentrations of carotenoids that serve as long-pass filters, narrowing the absorption bands of the visual pigments. See Hart (2001) and Cuthill (2005) for detailed tabulations of the data and discussions of the phylogenetic relationships and ecological correlates of these receptors.

Because in many species sexual selection has resulted in brightly colored males, birds have long been assumed to have good color vision, and the current premise is that color vision is mediated by the four classes of single cones. The pigeon has served as the principal subject for psychophysical studies of avian color vision (Wright 1979), providing the major source of quantitative behavioral data on saturation (Blough 1975), color boundaries (Wright and Cumming 1971), and hue discrimination (Wright 1972a, b). More recently, Palacios et al. (1990) and Palacios and Varela (1992) have studied color matches in the pigeon. This work was done before the presence, species distribution, and bimodal sensitivity of the UVS/VS cones were appreciated.

Recordings from optic tract of quail and ventral lateral geniculate nucleus of pigeons have revealed color opponent cells (Yazulla and Granda 1973; reviewed in Varela et al. 1993), a seemingly ubiquitous way of

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coding information about color. Furthermore, a model that is based on the premise that threshold color detection is determined by noise in receptors that in turn provide input to opponent processes (Vorobyev and Osorio 1998) has proved successful in describing behavioral data on discriminating color against a background, including by the budgerigar (Goldsmith and Butler 2003).

Aside from the pigeon, relatively little quantitative behavioral work has been done on avian color vision. Several studies show that sensitivity to UV is important in foraging and mate choice (reviewed in Cuthill et al. 2000). Two different behavioral paradigms indicate that domestic chicks (Osorio et al. 1999b) as well as European starlings and Japanese quail (Smith et al. 2002) use the UVS or VS receptor in opponency with one or more longer wavelength cones in making chromatic discriminations.

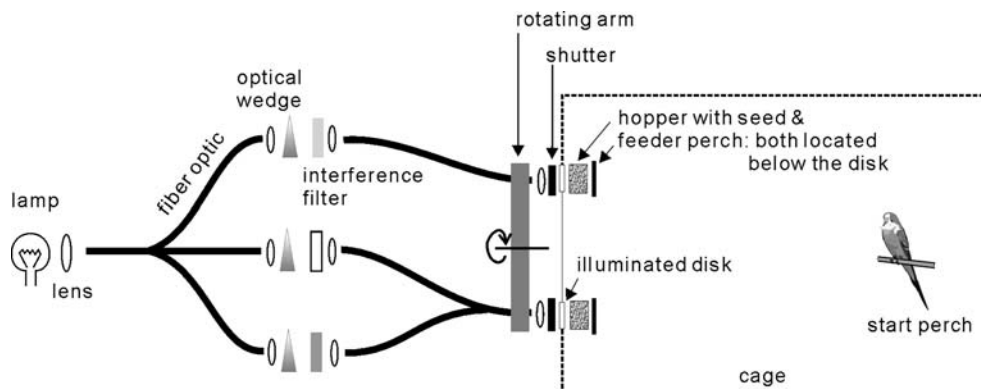
The present report describes how budgerigars are able to match hues in three different spectral regions, and how the results are quantitatively consistent with the spectral properties of the visual pigments. We find that the UVS cones are engaged as one of four receptors in a tetrachromatic color vision system involving at least three spectral opponent processes, although the full spectrum of terrestrial sunlight is required to activate all four classes of single cones simultaneously. The results are also consistent with the current view that double cones are not involved in color vision.

## Methods

### Birds

During the course of this work, which ran over several years, 14 birds contributed to the data. Budgerigars were

**Fig. 1** Diagram of the apparatus used to test the visibility of small amounts of UV light added to longer wavelengths and to find dichromatic hue matches for the birds.  $S^+$  (illuminated by the single fiber optic) appears at the top in this diagram—on the right from the perspective of the bird. The positions of  $S^+$  and  $S^-$  could be interchanged by rotating the horizontal bar to which the ends of the fiber optics were attached. Different wavelengths were selected by changing the interference filters. See the text for further description



obtained from local breeders. The birds were housed communally in the room with the test apparatus and were fed a commercial parakeet seed mix (Nature's Gold Premium, L&M Animal Farms, Pleasant Plain, OH, or similar blends, depending on availability) to which was added a commercial dietary supplement (Budgimine, Kellogg, Milwaukee, WI). This diet contained ample carotenoid to supply cone oil droplets and to maintain carotenoid-based color in the feathers. (Goldsmith and Butler 2003). During periods where the birds were working every day, most of their daily allotment of food came during the course of experimental runs, although some additional seed was given as a reward after a run was completed. Total daily intake was adjusted so as to maintain each bird's motivation to feed at the apparatus. The condition of each bird was assessed daily by observing its behavior and gently palpating the breast muscles. The tissue mass around the keel of the sternum was scored quantitatively and provided an index that was more accessible and useful than body weight, with which it correlated.

Best results were obtained with young birds that were initially habituated to the experimenter by hand feeding. Early training to the apparatus was facilitated by vocalization ("good," "no") with pitch modulation that could be interpreted by the birds as appeasement or threat (Morton 1977, 1982). All other reinforcement was positive. Inexperienced birds learned faster by being able to observe older birds work the apparatus.

### Apparatus

Light from a quartz-halogen lamp was delivered via a trifurcated fiber optic bundle to three sites (Fig. 1) where intensity and wavelength could each be varied independently. Narrow bands of wavelengths (12–15 nm half bandwidth) were selected with interference filters. For the UV, the filter (Wratten 18A) had a broader transmission band (consequences described below). Intensity was controlled by optical wedges consisting of thin films of inconel deposited on quartz disks in annuli of graded density. Pairs of counter-rotating disks were mounted in each wedge, thus making the absorbance spatially uniform in the local region where the light passed. The

absorbance of each wedge, as a function of the rotational position of the disks, was calibrated independently for each wavelength used. Reproducibility of wedge settings was about 0.02 absorbance units. Light fluxes were measured with a photodiode (UV100, United Detector Technology Sensors, Hawthorne, CA) whose absolute sensitivity had been calibrated through the spectrum.

After passing through the wedges and interference filters, one fiber optic path was reserved for the stimulus ( $S+$ ) to which the birds were trained. The remaining two light paths were recombined with a bifurcated fiber optic bundle to form the comparison stimulus ( $S-$ ). The output ends of these two fiber optic paths,  $S+$  and  $S-$ , illuminated stimulus windows that were viewed by the birds from the opposite side. Each window was a 1-cm disk of frosted quartz that diffused the light from the fiber optic bundles. The terminations of these two fiber optic bundles were mounted at opposite ends of a rotating arm. Starting from a horizontal position, the arm could be rotated  $180^\circ$  around its midpoint in a vertical plane, thereby interchanging the reference and comparison lights at the two windows. A pair of shutters was mounted immediately behind each stimulus window. A covered food hopper and a feeding perch were located in front of and under each stimulus window. Shutters, wedges, and the position of the rotating arm were controlled with stepping motors from the computer running the experiment. The presence of the bird at any of the perches altered signals from light-emitting diodes. The positions of the bird (on which perch or in flight), wedges, shutters, and other features of the equipment were displayed on the computer's monitor during the course of an experiment (programmed on LabView 6.1, National Instruments Corp.).

During experiments, a bird viewed the two stimulus windows simultaneously from a start perch at a distance of 0.42 m. The windows were positioned horizontally 16 cm apart, and when viewed from the start perch, each subtended an angle of about  $1.4^\circ$ . The shutters opened only after the light bar was in the proper horizontal position, the wedges had been set, and the bird was on the start perch. Upon appearance of the stimuli, the bird moved to the perch under the stimulus window it had selected. A correct choice caused the hopper to open, permitting a 3-s access to food. An incorrect choice closed both shutters, and no food was available. A run with an individual bird consisted of 30 trials and took 15–30 min, depending on the difficulty of the comparisons.

### Setting the task

The birds were trained to associate food with a monochromatic light,  $S+$ . The comparison stimulus,  $S-$ , was usually a mixture of two wavelengths. The proportion of the two wavelengths at  $S-$  was varied from trial-to-trial, but the total quantum flux ( $I_{S-}$ ) was kept constant. The

quantum flux at  $S+$  ( $I_{S+}$ ), however, was changed from trial-to-trial in a quasirandom manner over a range of  $\pm 0.2$  or  $\pm 0.3$  log units and in a way that was independent of the changes in spectral content of  $S-$ . Over time, all combinations of comparisons were presented to the birds with approximately equal frequency. By varying the intensity of  $S+$  during both training and testing, the effect of brightness on hue discriminations could be assessed. In a series of experiments explicitly designed to test intensity discrimination, spectral content of  $S+$  and  $S-$  was identical.  $I_{S+}$  was held constant and  $I_{S-}$  was varied.

The appearance of  $S+$  at the left or right window also varied in quasirandom fashion. With sequences of more than three consecutive appearances of  $S+$  at the same window or with sequences of very difficult comparisons, the birds frequently began to bias their responses to one stimulus window. Sequences were therefore designed to minimize this side-bias, and the sequence of presentations was resident in the computer when a run began. Further details of the apparatus can be found in Goldsmith and Butler (2003).

### Spectra of the visual pigments

The budgerigar has four cone pigments with  $\lambda_{\max}$  at 370, 445, 508, and 565 nm as shown by microspectrophotometry (Bowmaker et al. 1997). There are correspondingly four spectral classes of single cones. The double cones contain P565. Spectra down to 300 nm were computed using the approaches of Stavenga et al. (1993) and Lamb (1995), supplemented by information from Palacios et al. (1996) on the position of the  $\beta$ -band in the near UV. The receptors are small, and self-screening was ignored. The screening effects of the cone oil droplets were computed from the absorbance spectra of carotenoids that have been identified in avian cone oil droplets (Goldsmith et al. 1984), work that involved microspectrophotometric measurements of single droplets that had been expanded by fusion with larger droplets of mineral oil. Identification was supplemented and cleaner spectra obtained following high-performance liquid chromatography of retinal extracts of chickens (unpublished observations). The spectral response of a cone screened by an oil droplet was calculated as  $\alpha(\lambda) = 10^{-d \cdot C(\lambda)} \cdot P(\lambda)$ , where  $d$  is the peak absorbance of the droplets in vivo (calculated from the sizes of the expanded and native droplets),  $C(\lambda)$  is the normalized absorbance spectrum of the carotenoid or mixture of carotenoids in the droplet, and  $P(\lambda)$  is the normalized absorbance of the visual pigment.

### Predicting hue matches from the spectral properties of the photoreceptors

In what follows, hue refers to the sensation of color as it varies with the wavelength composition of the stimulus. The stimuli were luminous windows displayed on a dark,

achromatic background, and contrast effects were ignored. Our operating assumption is that only the single cones are involved in avian color vision. Regardless of the complexities present in the afferent path, two stimuli will appear the same if they stimulate the array of retinal cones in the same way.

The sensation of brightness depends on the relative magnitude of quantum catch, thus it varies with intensity of the stimulus, again ignoring contrast affects. It is likely that the signals generated by both single and double cones vary in brightness, but the double cones generate an achromatic signal. How single and double cones interact is unknown. Whether convergent input from single cones generates an achromatic signal, as in human color vision, is an open question.

The quantum catch of monochromatic light by a cone pigment,  $P_i$ , depends on  $I(\lambda) \cdot \alpha_{P_i}(\lambda)$ , where  $I(\lambda)$  is the quantum flux at wavelength  $\lambda$  and  $\alpha_{P_i}(\lambda)$  is the absorption coefficient of  $P_i$  at wavelength  $\lambda$ , after accounting for screening by the oil droplet. In a spectral region where only two receptors are active, a dichromatic hue match is possible. The two receptor pigments can be designated  $P_l$  and  $P_s$ , indicating that one is maximally sensitive at longer wavelengths than the other. The condition for a hue match is that  $P_s$  and  $P_l$  be stimulated in the same ratio by the training wavelength ( $S+$ ) as they are by a mixture of two other wavelengths in a comparison target ( $S-$ ). Stated algebraically,

$$\frac{\alpha_{P_s}(\lambda_{S+})}{\alpha_{P_l}(\lambda_{S+})} = \frac{\alpha_{P_s}(\lambda_{S-}^1) + q \cdot \alpha_{P_s}(\lambda_{S-}^2)}{\alpha_{P_l}(\lambda_{S-}^1) + q \cdot \alpha_{P_l}(\lambda_{S-}^2)}, \quad (1)$$

where  $\alpha_{P_i}$  is the spectral absorption at wavelength  $\lambda$ ,  $q = \frac{I(\lambda_{S-}^2)}{I(\lambda_{S-}^1)}$  (the ratio of intensities of the two wavelengths in the  $S-$  mix at match), and  $\lambda_{S-}^1 < \lambda_{S+} < \lambda_{S-}^2$ . Note that terms in the numerator of (1) refer to  $P_s$  and those in the denominator to  $P_l$ . Rearranging,

$$q = \frac{\alpha_{P_s}(\lambda_{S-}^1) \cdot \alpha_{P_l}(\lambda_{S+}) - \alpha_{P_s}(\lambda_{S+}) \cdot \alpha_{P_l}(\lambda_{S-}^1)}{\alpha_{P_s}(\lambda_{S+}) \cdot \alpha_{P_l}(\lambda_{S-}^2) - \alpha_{P_s}(\lambda_{S-}^2) \cdot \alpha_{P_l}(\lambda_{S+})}. \quad (2)$$

With knowledge of the absorption spectra of the visual pigments, Eq. (2) predicts the ratio of intensities of the two wavelengths at  $S-$  that are required for matching the hue of a training wavelength at  $S+$ .

#### Computing relative quantum catch in hue discriminations

Each nominal wavelength in Eq. 2 actually encompasses the band pass of the associated filter. For narrow-band interference filters, using just the peak wavelength introduces a small but significant error, and for the UV filter (Wratten 18A) the issue is even more important. Therefore, for each filter the normalized spectral distribution of quantum flux,  $\frac{Q(\lambda)}{Q_{\max}}$ , was computed at 1-nm intervals as  $Q(\lambda) = T(\lambda) \cdot I(\lambda)$ , where  $T(\lambda)$  is the transmis-

sion spectrum of the filter at wavelengths where  $T > 0.1\%$  of the peak transmittance, and  $I(\lambda)$  is the relative quantum flux over the same wavelength band, calculated as black body radiation at the color temperature of the lamp. The relative excitation of each pigment by each stimulating light was then computed as  $\bar{\alpha}_{S,P} = \sum \frac{Q(\lambda) \cdot \alpha(\lambda)}{Q_{\max}}$ , where  $\alpha(\lambda)$  is the absorption coefficient of the visual pigment (including any effect of the cone oil droplet), and the summation occurs across the transmission band of the filter. Recasting Eq. 2 in terms of these summations:

$$q = \frac{\bar{\alpha}_{S-(1),P_s} \cdot \bar{\alpha}_{S+,P_l} - \bar{\alpha}_{S+,P_s} \cdot \bar{\alpha}_{S-(1),P_l}}{\bar{\alpha}_{S+,P_s} \cdot \bar{\alpha}_{S-(2),P_l} - \bar{\alpha}_{S-(2),P_s} \cdot \bar{\alpha}_{S+,P_l}}, \quad (3)$$

which is the form used in analyzing hue matches.

#### Analysis of frequency of correct choice

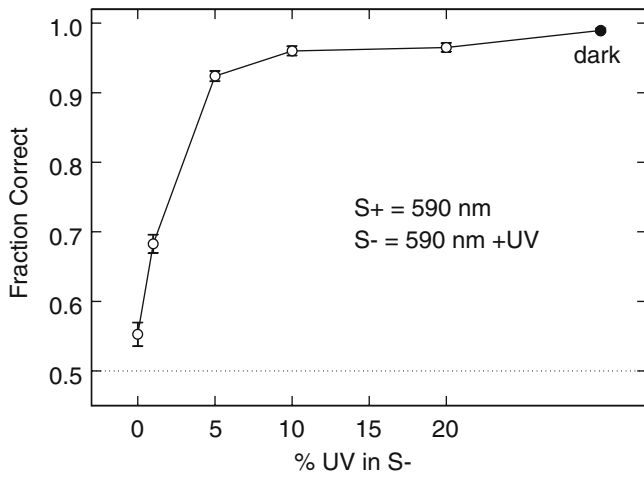
The fraction of choices that were correct ( $f$ ) was tabulated for various combinations of wavelengths and intensities (quantum flux). The standard deviation of the mean of individual data points was calculated as  $\sqrt{\frac{f(1-f)}{n}}$ , where  $n$  is the number of choices. In discriminating an illuminated from an unilluminated disk,  $f$  varied as a function of  $\log I$  along a sigmoid curve that is described by logistic regression, ranging from 0.5 (chance performance  $\pm$  random error) to 1 (Goldsmith and Butler 2003). In the present experiments on hue discrimination, intensity was varied at  $S+$  and kept constant at  $S-$ . The statistical issue is whether discrimination of a particular pair of hues is perturbed by differences in the intensity at  $S+$ , i.e., whether the slope of the logistic regression of  $f$  on  $\log(I_{S+}/I_{S-})$  is significantly different from 0. The probabilities reported in the results (2-tailed) are based on  $t$  statistics resulting from regression analyses (software from S-PLUS, Insightful Corp.).

## Results

The presence of UV alters the appearance of long wavelength stimuli

The birds could readily distinguish yellow light from the same wavelength to which small amounts of UV had been added. For example, Fig. 2 shows for  $S+ \equiv 590$  nm how the fraction of choices that were correct ( $f$ ) increased sharply with increasing percentage of UV in the (UV + 590 nm) mix that was present at  $S-$ . For each data point in this figure, values of  $f$  were averaged across all ratios of intensity.

Figure 3 shows data broken out by individual bird, plotted as fraction correct as a function of the log of the intensity ratio,  $I_{S+}/I_{S-}$ . The open symbols are for  $S+ \equiv (590$  nm) and  $S- \equiv (10\%$  UV + 90% 590 nm). The birds selected  $S+$  with frequency  $f = 0.96 \pm 0.0067$



**Fig. 2** Pooled data for 6,317 observations on six birds showing how the addition of small proportions of UV to 590 nm yellow allows the stimulus to be discriminated from pure 590 nm. Error bars are  $\pm 1$  SE. Data averaged across all ratios of intensities ( $I_{S+}/I_{S-}$ ). A small effect of relative intensity lifts the point at 0% UV significantly above chance. The point labeled “dark” represents the absence of any visible illumination behind the  $S-$  window

SE, and there was no consistent effect of varying the relative intensity of  $S+$  over  $\pm 0.3$  log units. However, when  $S+ \equiv S- \equiv (590 \text{ nm})$  (no UV present, data plotted as filled symbols),  $f$  scattered widely around chance (0.5). Although for  $S+ \equiv S-$  three of the birds (right column of panels) seemed to be cuing on the brighter of the two stimulus disks, this effect became clearer by aggregating the data. Figure 4 shows pooled

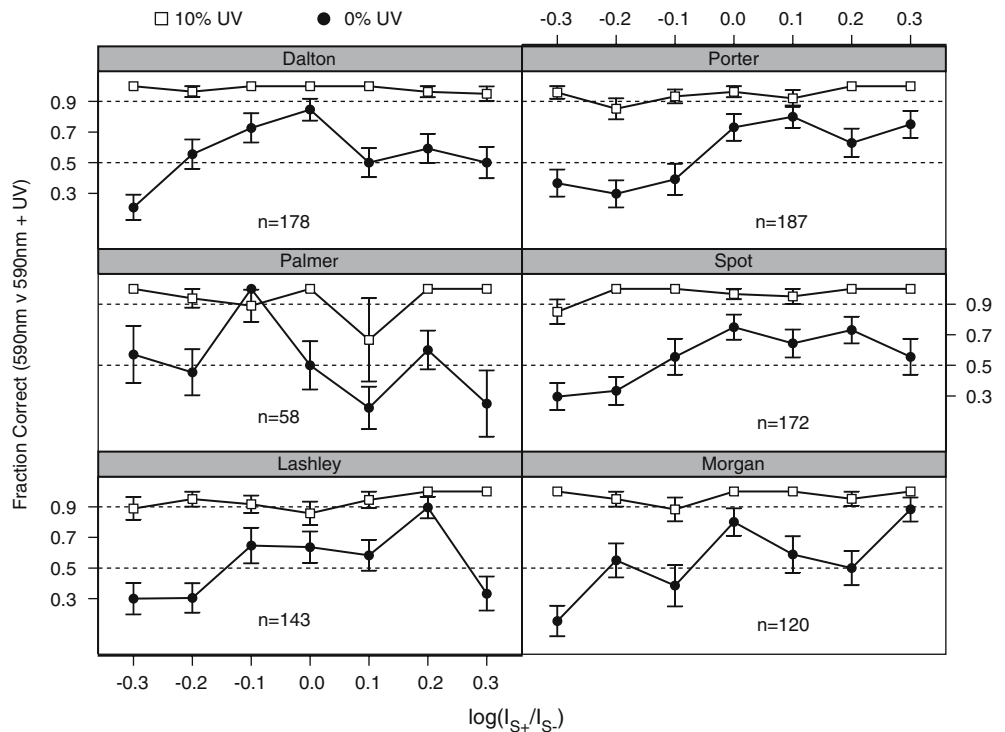
data for all six birds and for several other mixes of (UV + 590 nm) in  $S-$ . For four of the curves [ $S- \equiv (0, 1, 5,$  and  $10\% \text{ UV} + 590 \text{ nm})$ ], the positive slopes of the logistic regressions are significantly different from 0 ( $P < 0.001, n = 856; P < 0.001, n = 1,232; P = 0.001, n = 1,273,$  and  $P = 0.01, n = 846,$  respectively). However, the presence of more than 1% UV in  $S-$  was much more important than the relative intensity of  $S+$  in influencing choices (top four curves in Fig. 4).

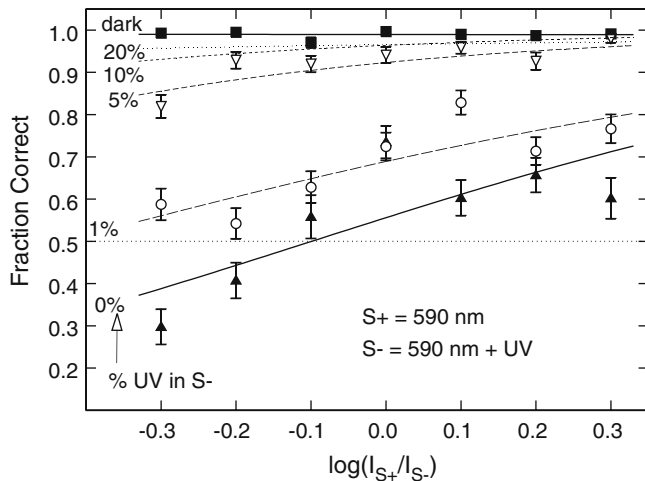
In the absence of a chromatic cue (no UV in  $S-$ ), most of the scores fell below chance for  $I_{S+} < I_{S-}$  and rose above chance for  $I_{S+} > I_{S-}$ . Averaged across all values of  $\log(I_{S+}/I_{S-}), f = 0.55 \pm 0.017 \text{ SE}$  (Fig. 2). This value therefore appears to be significantly above chance because when discriminations based on hue were difficult or unreliable, choices were influenced by differences in intensity. This issue recurs in different form throughout the results.

The presence of UV also alters the appearance of 440 nm stimuli, but the salience of intensity can vary

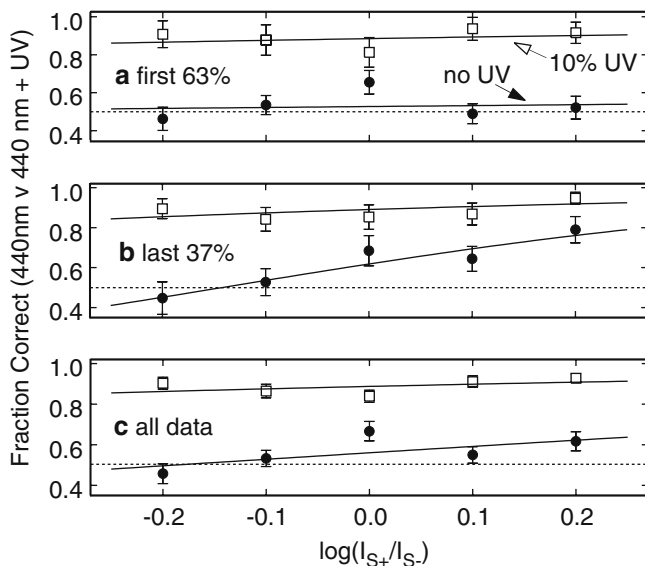
The birds readily discriminated 440 nm from (10% UV + 90% 440 nm) (Fig. 5, open symbols). Data in the upper panel were obtained immediately after training and represent the first 63% of the test data. Average fraction correct (open circles, all panels) was 0.89 (statistic for the bottom panel,  $\pm 0.012 \text{ SE}, n = 614$ ). Similarly, there was no effect of intensity—the variation of  $f$  with  $\log(I_{S+}/I_{S-})$  was not statistically significant ( $P > 0.2$  in each panel, A:  $n = 384,$  B:  $n = 230,$  C:  $n = 614$ ).

**Fig. 3** Frequency of correct choices for six birds trained to 590 nm and tested against a mixture of (90% 590 nm + 10% UV) (open symbols) or (100% 590 nm) (filled symbols). The intensity of the training light ( $I_{S+}$ ) was varied by  $\pm 0.3$  log units relative to the comparison light (abscissa). Horizontal dashed lines indicate fractions correct of 0.5 (chance) and 0.9. Error bars are  $\pm 1$  SE. The number of choices for  $S- \equiv (100\% \text{ 590 nm})$  is given by  $n$  for each bird; the numbers of observations for  $S- \equiv (90\% \text{ 590 nm} + 10\% \text{ UV})$  were similar





**Fig. 4** Effect of the relative intensity of  $S+$  relative to  $S-$  on the fraction of correct choices for several different proportions of UV in  $S-$ . Pooled data from six birds and 6,312 individual choices. Error bars are  $\pm 1$  SE. Horizontal dotted line indicates chance performance. Note that at this long wavelength, the salience of intensity was greatest when the discrimination of hue was most challenging



**Fig. 5** Discrimination of 440 nm from (440 nm + UV) plotted versus the log of the intensity ratio of  $S+$  and  $S-$ . Open squares  $S- \equiv (90\% \text{ 440 nm} + 10\% \text{ UV})$ . Filled circles  $S- \equiv (440 \text{ nm})$ . **a** a subset of the data consisting of the first 63% of the measurements. **b** the final 37% of the data. **c** all data combined. The birds appeared to cue on relative intensity only in the absence of UV and after extended experience with the task (**b**). Moreover, the net effect was not as pronounced as at 590 nm (compare **c** with Fig. 4). Data points are averages of  $f \pm 1$  SE. Solid curves are logistic regressions. The slopes of the lower curves (filled circles) in panels **b** and **c** are significantly different from 0 ( $P < 0.001$  and  $P = 0.032$  respectively); all other regressions are statistically flat. Further statistical details are in the text. Dashed lines indicate chance performance

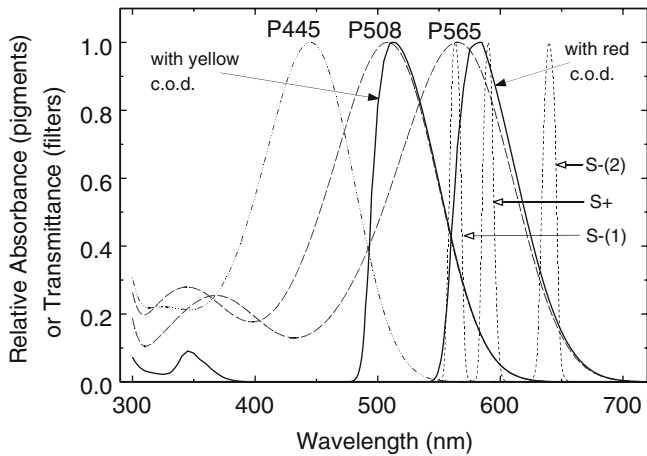
The controls—no UV present,  $S+ \equiv S- \equiv 440 \text{ nm}$  (Fig. 5, filled circles)—require further consideration. In panel A, correct choices scattered around  $f=0.5$  and were uninfluenced by the relative intensities of  $S+$  and  $S-$ . (Slope of the logistic regression is statistically flat;  $P=0.45$ ,  $n=385$ ). Thus, during this period of testing, intensity differences had no salience for the birds. This observation provides an important instrumental control for the experimental equipment. That performance fell to chance when  $S+ \equiv S-$  and the birds ignored intensity differences indicates that they were not receiving spurious cues about the position of  $S+$  independently of the chromatic content and intensity of the stimulus.

As testing continued, however, in the absence of a chromatic cue, the birds became influenced by the brighter of the two stimuli (Fig. 5, middle panel, filled circles), as they had been at longer wavelengths. The slope of the logistic regression is significantly different from 0 ( $P < 0.01$ ,  $n=228$ ). In the aggregated data set (bottom panel of Fig. 5) the effect of intensity is small (filled circles,  $P=0.032$ ,  $n=613$ ).

In summary, the presence of small proportions of UV changes the color of 440 nm just as effectively as it does for 590 nm. Second, the salience of intensity in difficult chromatic discriminations appears to vary in different spectral regions. Third, when  $S+$  and  $S-$  are both set to the training wavelength, departures from chance performance can be caused by differences in  $I_{S+}$  relative to  $I_{S-}$ .

#### A hue match at long wavelengths

At long wavelengths there should be a region of the spectrum where only two of the visual pigments in single cones are absorbing significantly and a dichromatic hue match should be possible (Fig. 6). The yellow cone oil droplets (c.o.d.) associated with P508 and the red droplets found with P565 in single cones have very high absorbance (Goldsmith et al. 1984) and function as long-pass filters, narrowing the spectral sensitivity of the visual pigments (heavy, solid curves in Fig. 6). At 560 nm, sensitivity of P445 has fallen to a low value, and a hue match was sought at longer wavelengths by training the birds to  $S+ \equiv 590 \text{ nm}$  and testing them against  $S- \equiv (563 + 639 \text{ nm})$ . The quantal content in the mixture at  $S-$  was kept constant as the proportions of 563 and 639 nm were varied, and  $\log(I_{S+}/I_{S-})$  was varied over 0.5 log units by adjusting the intensity of  $S+$ . With eight different chromatic mixtures, six intensity ratios, the left/right change of the position of  $S+$ , and the participation of several birds, many individual choices were required to accumulate a statistically meaningful database. Results for seven birds are shown in Fig. 7, where fraction correct ( $f$ ) is plotted as a function of the chromatic mix in  $S-$  (averaging across all values of  $I_{S+}/I_{S-}$ ). The top panel in the right column shows the average for all seven birds. All of the birds



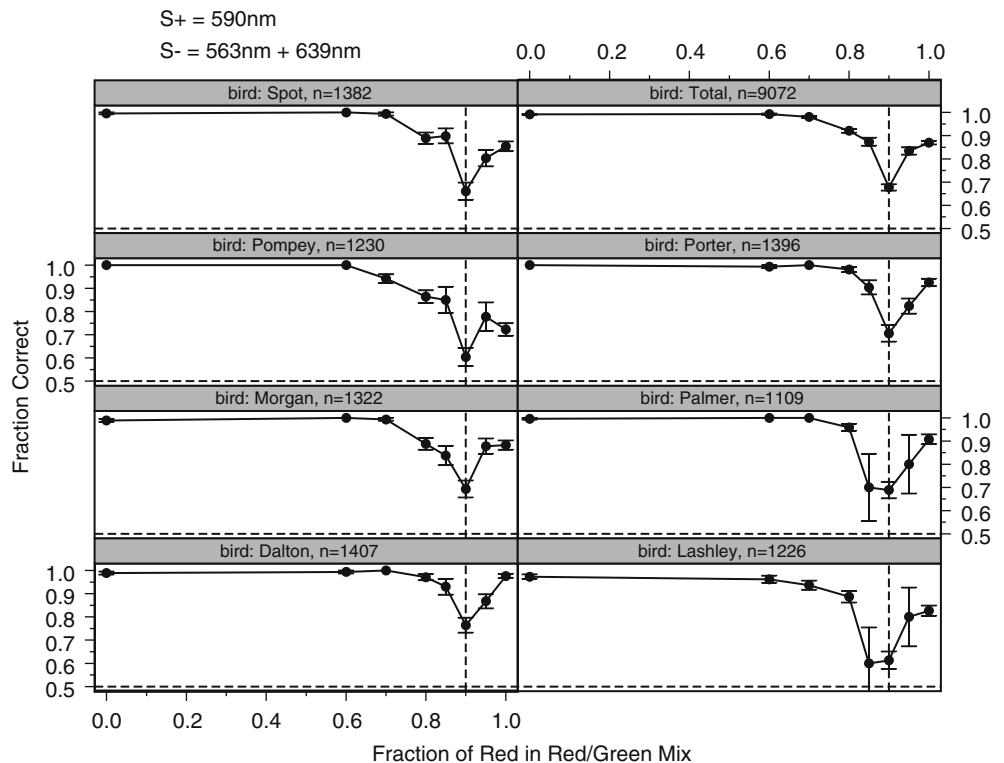
**Fig. 6** Absorbance spectra of three of the budgerigar's four cone pigments (P445, P508, and P565) are shown by the *broad dashed curves*. Note that all have significant absorption by the  $\beta$ -band at 300–400 nm. *Heavy unbroken curves* show the effects of screening by cone oil droplets on two of the visual pigments. Sensitivity of P508 at wavelengths shorter than about 480 nm is greatly reduced by the yellow cone oil droplets (for purposes of calculation peak absorbance = 8; carotenoid composition 10% C385 and 90% common xanthophyll, half of which was a *cis* isomer). Similarly, the even greater density of astaxanthin in the red oil droplets (absorbance = 20) renders the single cones containing P565 insensitive at wavelengths shorter than about 540 nm. Values of absorbance are consistent with measurements on expanded droplets (Goldsmith et al. 1984). The *three narrow dotted curves* are transmission spectra of the filters used for finding a dichromatic hue match (563, 590, and 639 nm)

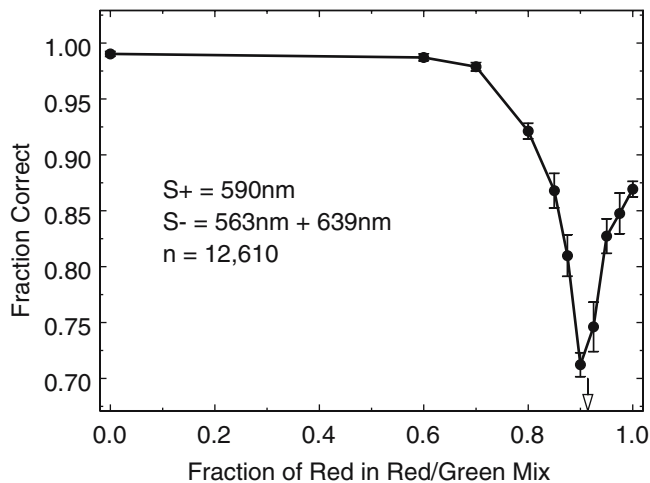
showed a minimum in the curve (point of greatest confusion) when  $S^- \equiv (90\% \text{ 639 nm} + 10\% \text{ 563 nm})$ . The depth of the minimum varied among the birds, and the average was  $f = 0.68 \pm 0.056 \text{ SE}$ , significantly above chance.

Figure 8 summarizes all of the data for this experiment, combining the 9,072 choices in the upper right panel of Fig. 7 with another 3,538 individual choices by these and other birds. The lowest average number of correct choices ( $f = 0.71 \pm 0.01$ ) occurred at 90% 639 nm. Note that the birds were better at discriminating 563 from 590 nm (0 red in the red/green mix,  $f = 0.99 \pm 0.002$ ) than they were at discriminating 639 from 590 nm ( $f = 0.87 \pm 0.007$ ).

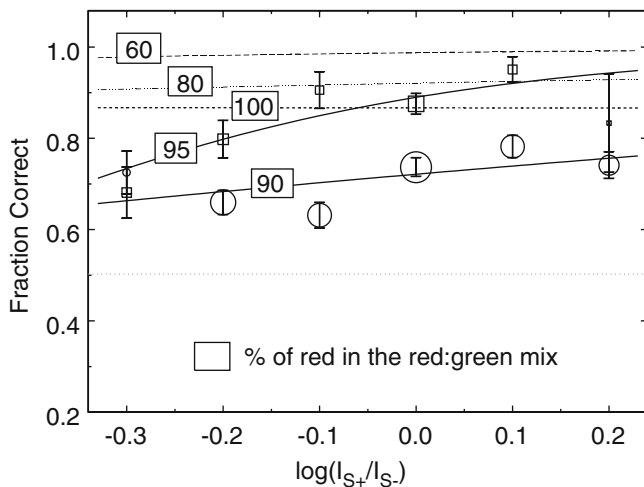
Figure 9 shows how the fraction of correct choices was influenced by  $\log(I_{S+}/I_{S-})$  for several mixes of red and green. Only at mixtures close to and on the red side of a hue match ( $S^- \equiv 90\text{--}95\% \text{ 639 nm}$ ) did the regression functions have a statistically significant positive slope (Table 1). For all other mixtures, three of which are shown in Fig. 9 by the thin, broken regression lines (60, 80, and 100% 639 nm, data points omitted for clarity), fraction correct did not vary significantly with the relative intensities of  $S+$  and  $S-$ . These data are consistent with results in the two preceding sections: In experiments where the birds were trained to a specific color, differences in intensity between  $S+$  and  $S-$  influenced choices only under conditions in which hue discrimination was difficult.

**Fig. 7** Average results for seven individual birds in a dichromatic match of yellow,  $S+ \equiv (590 \text{ nm})$ , with a mixture of red and green,  $S- \equiv (639 + 563 \text{ nm})$ . The fraction of choices that were correct was smallest for  $S^- \equiv (90\% \text{ 639} + 10\% \text{ 563})$ . The number of choices on which each curve is based on given next to the bird's name





**Fig. 8** Pooled data for all birds in the long-wavelength hue match of Figs. 6 and 7. Arrow at 0.915 on the abscissa indicates the match point that was calculated from spectra of the visual pigments



**Fig. 9** Variation of fraction correct with  $\log(I_{S+}/I_{S-})$  for several percentages of red present at  $S^-$  in the long-wavelength hue match. Only at mixtures close to the match did intensity differences have salience for the birds, here indicated by a positive slope of the logistic regression at 90% and 95% 639 nm. Error bars are  $\pm 1$  SE; bubbles are approximate indicators of the relative number of observations at each point. See Table 1 for a summary of statistical significance of the slopes for these and other mixes of the red and green lights at  $S^-$

**Table 1** Discrimination of 590 nm from mixes of 639 + 563 nm: logistic regression of  $f$  on  $\log \frac{I_{S+}}{I_{S-}}$

% 639 nm at $S^-$	Positive slope	Significance	$n$
80	No	—	1,472
85	No	—	477
87.5	No	—	447
90	Yes	$P < 0.01$	1,779
92.5	Yes	$P < 0.01$	386
95	Yes	$P < 0.01$	602
97.5	No	—	387
100	No	—	2,287

With  $S^+ \equiv 590$  nm and  $S^- \equiv (563 + 639)$  nm, the condition for a match is given by

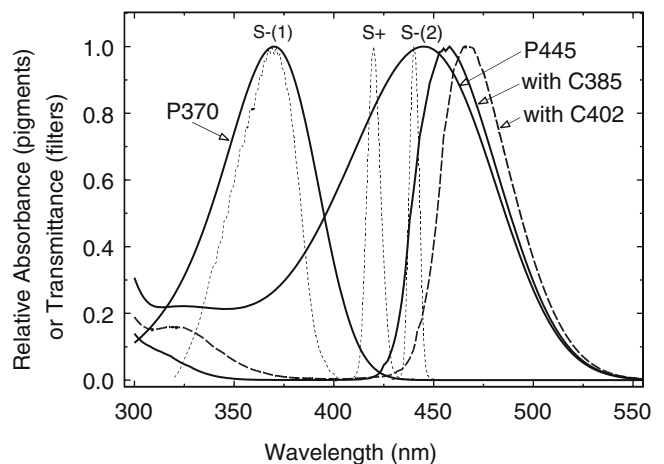
$$q = \frac{\bar{\alpha}_{S^-(563),P508} \cdot \bar{\alpha}_{S^+(590),P565} - \bar{\alpha}_{S^+(590),P508} \cdot \bar{\alpha}_{S^-(563),P565}}{\bar{\alpha}_{S^+(590),P508} \cdot \bar{\alpha}_{S^-(639),P565} - \bar{\alpha}_{S^-(639),P508} \cdot \bar{\alpha}_{S^+(590),P565}}, \quad (3a)$$

in which  $q = I(639)/I(563)$  present at  $S^-$  at match. Using the absorbance spectra of P508 and P565 shown by the heavy curves in Fig. 6 and integrating across the bandwidth of the filters to find values of  $\bar{\alpha}_{S,P}$  (Methods), the proportion of red in the mixture of red and green at match is  $q/(1+q) = 0.915$ , in excellent agreement with the results of the behavioral experiment (Fig. 8, short arrow above the abscissa).

The effect of the cone oil droplets on this calculation is very small: the predicted match point for unscreened cone pigments was  $q/(1+q) = 0.90$ . This small difference is caused by screening effects on P565 because both comparison and training wavelengths are located so far onto the long-wavelength limb of the absorption spectrum of P508 that the effects of the yellow oil droplets are inconsequential. The role of red and yellow oil droplets is therefore to restrict sensitivity of the LWS and MWS cones at short wavelengths.

#### A hue match at short wavelengths

The screening of P508 and P565 by yellow and red oil droplets greatly reduces if not eliminates sensitivity of these cones in the violet and UV region of the spectrum (Fig. 6). At short wavelengths, therefore, color vision should depend entirely on PUV and P445, as suggested by Fig. 10. To test this possibility, birds were trained to



**Fig. 10** Spectral distributions of the lights used at  $S^+$  and  $S^-$  (thin dashed curves) in the short-wavelength hue match, and normalized spectra of P370 and P445. For P445, two possible effects of screening by cone oil droplet are also shown: the carotenoid galloxanthin with  $\lambda_{\max} = 402$  nm (C402) at a peak absorbance of 2.5, and a carotenoid with maximal absorption in the near UV (C385) with peak absorbance of 3.5

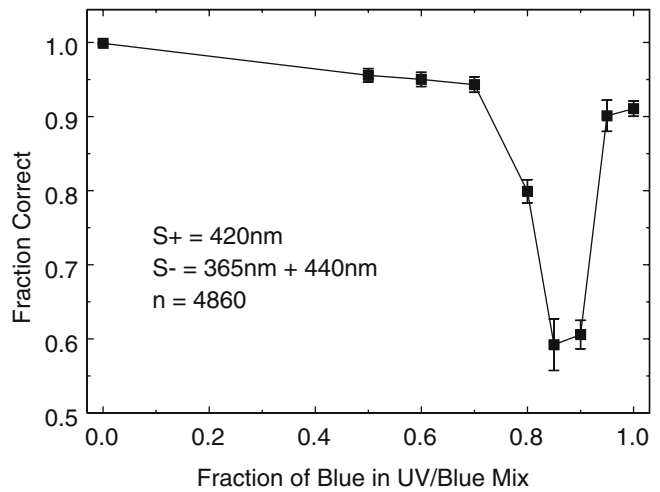


$S+ \equiv 420 \text{ nm}$  and tested against mixtures of (UV + 440 nm). Spectral distributions of the three lights are shown by the thin dashed lines in Fig. 10.

For five of the six birds used in this experiment, there was a minimum in the discrimination curve when  $S-$  contained 85–90% 440 nm (Fig. 11). The data for one bird (Dalton) failed to show a single clear minimum; the likely reason will be considered later. Aggregated data for the other five birds are shown in Fig. 12. We interpret the minimum in Fig. 12 to approximate a hue match, and that at short wavelengths (violet and ultraviolet), budgerigars are dichromatic. The birds were adept in discriminating UV from 420 nm ( $f=1$ ), and performance fell only slightly for the comparison of 440 with 420 nm ( $f=0.91 \pm 0.01 \text{ SE}$ ).

Figure 13 shows the fraction of correct choices as a function of  $\log(I_{S+}/I_{S-})$  for several spectral mixtures at  $S-$ . The slopes of the logistic regressions are statistically flat at these and the other mixes in the experiment of Fig. 12, indicating that intensity differences had no salience in this experiment. This result differs from that observed in the long-wavelength hue matches, where the birds were influenced by differences in intensity when discriminations were difficult.

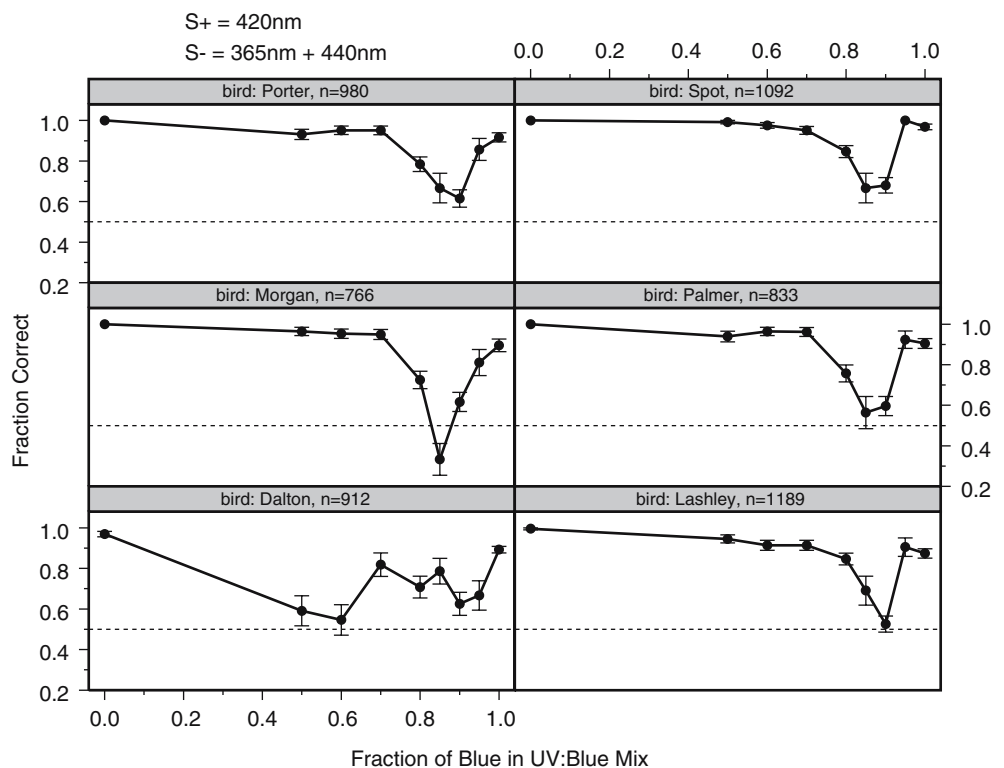
This hue match also differs from that at long wavelengths in having a deeper minimum; for three of the birds  $f$  fell into chance performance (Fig. 11), and the average was  $0.59 \pm 0.035 \text{ SE}$  (Fig. 12) compared with  $0.71 \pm 0.01$  at long wavelengths. Heretofore, we have argued that departures from chance performance in hue matches can be attributed to averaging  $f$  over all ratios



**Fig. 12** Pooled data (4,860 individual choices) for five of the birds in Fig. 11, showing a hue match for 420 nm with a mixture of (85–90% 440 nm + 15–10% UV). Error bars are  $\pm 1 \text{ SE}$

of intensity at  $S+$  and  $S-$ . In dichromatic matches, however, two additional factors may be present: the discrete mixtures of  $\lambda$  that were used at  $S-$  may not have included the best match, or  $S-$  may appear less saturated than  $S+$ . (In human psychophysics, if the mixture of wavelengths at  $S-$  contributes more to a sensation of “whiteness” than the single wavelength at  $S+$ , the hue of  $S-$  will appear somewhat desaturated.) We have no data on possible differences in saturation (or even the applicability of inferences about saturation based on

**Fig. 11** Hue matches for six birds trained to 420 nm and tested against mixtures of (365 + 440 nm). One bird (Dalton) failed to show a clear match. Data averaged over all intensities



human color vision), but Fig. 12 indicates that the match point was actually between 85 and 90% 440 nm. With a more precise mixture at  $S-$ , the average value of  $f$  at the match might well have been even closer to 0.5.

In this test the conditions for a match are

$$q = \frac{\bar{\alpha}_{S-(UV),P370} \cdot \bar{\alpha}_{S+(420),P445} - \bar{\alpha}_{S+(420),P370} \cdot \bar{\alpha}_{S-(UV),P445}}{\bar{\alpha}_{S+(420),P370} \cdot \bar{\alpha}_{S-(440),P445} - \bar{\alpha}_{S-(440),P370} \cdot \bar{\alpha}_{S+(420),P445}} \quad (3b)$$

For this experiment, however, calculating values of  $\bar{\alpha}_{S,P}$  requires two decisions. First, the  $\lambda_{max}$  of the UV cone is reported at 365 (Wilkie et al. 1998) and 370 nm (Wilkie et al. 2000). Second, the absorbance of P445 is altered by cone oil droplets that appear colorless but absorb at very short wavelengths (Bowmaker et al. 1997; J.K. Bowmaker, personal communication). The principal carotenoid is likely galloxanthin ( $\lambda_{max}$  402 nm, hereafter referred to as C402), although some avian cone oil droplets also contain a carotenoid absorbing at still shorter wavelengths, C385 (Goldsmith et al. 1984). For purposes of computation we assume C385 has an absorption that is similar in shape to the better-characterized spectrum of C402. Calculated effects of these oil droplets on normalized spectra of P445 are shown in Fig. 10. Spectra of some of the carotenoids present in cone oil droplets can be found in Goldsmith and Butler (2003).

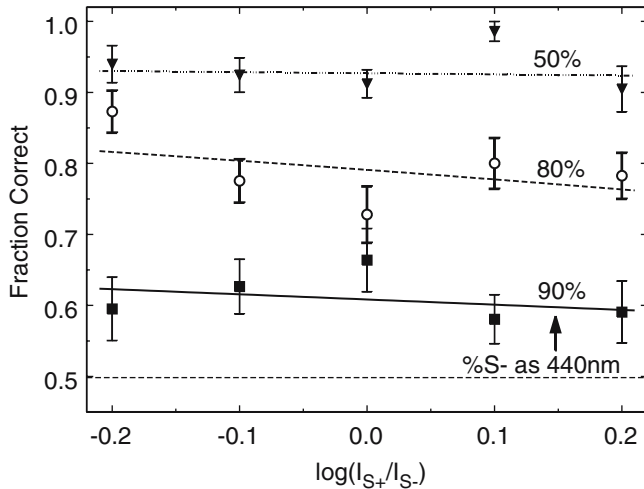
Figure 14 shows calculations of the expected match of 420 nm with a mixture of near UV and 440 nm, plotted as functions of the peak absorbance of the oil drop in the 445 nm cones. The calculations are made for the UV pigment with  $\lambda_{max}$  at 370 (filled circles) and repeated for a UV pigment with  $\lambda_{max}$  at 365 nm (open circles). The calculations also make two different

assumptions (stated in Fig. 14) about the carotenoid content of the cone oil droplets. These calculations also assume that P508 and P565 are not active in this short-wavelength region, as shown in Fig. 6. (As discussed later, the UV leakage of yellow oil drops shown in Fig. 6 may be exaggerated.) If P445 were unscreened by an oil droplet and the UV cone contained P370, the match should occur with  $q/(1+q) > 0.98$  (Fig. 14, 0 on the abscissa). With increasing absorbance of the oil droplet, the necessary fraction of 440 nm in the mix at  $S-$  decreases (curves descending in arcs from the convergence point at their upper ends). This analysis provides an estimate of the peak absorbance in the oil droplets where the curves pass through the shaded box delineating the range in which the match was observed experimentally. This estimate of 2.5–4.2 is independent of but overlaps with direct physical measures on other species, made by expanding the native droplets (Goldsmith et al. 1984).

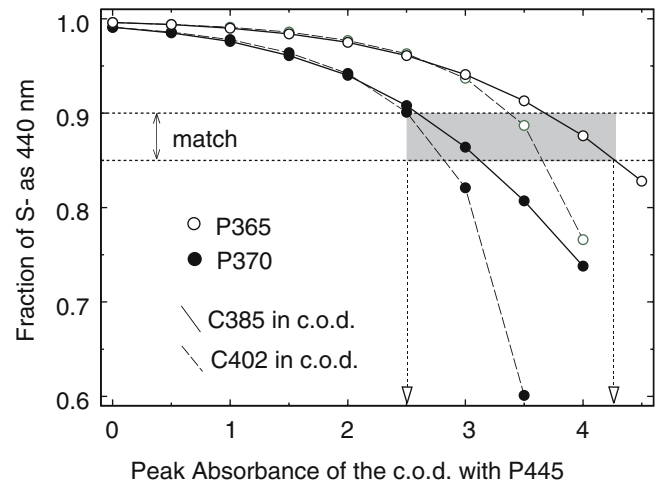
### A dichromatic match in midspectrum

If color vision is mediated by the single cones, there should be a midspectral region (440–540 nm), in which only two pigments, P445 and P508, are active. An experiment was undertaken to see if  $S+ \equiv 502$  nm could be matched by  $S- \equiv (461 + 520$  nm). These three wavelengths should generate very little excitation of the single cones containing either P565 or P370 (Fig. 15)

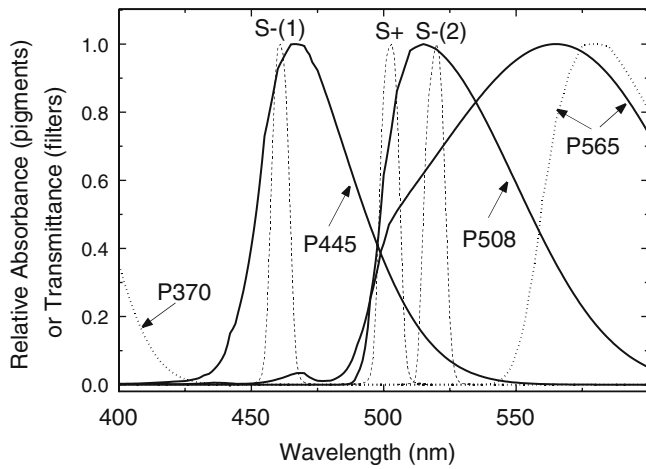
Figure 16 shows the results broken out by bird. There is a suggestion of a minimum at about 30% 461 nm in the mix, but compared with the results at both longer



**Fig. 13** No salience of relative intensity in a hue match involving UV and violet lights, based on a subset of the data in Fig. 12. Fraction correct as a function of the log of the relative intensities of  $S+$  and  $S-$  for three mixes of UV and 440 nm in  $S-$ . Regression slopes are statistically flat for these and the other mixes that are not shown



**Fig. 14** Calculated mixture of 440 nm and UV lights required to match the 420 nm training light, plotted as a function of the absorbance of the cone oil droplet in front of P445. The oil droplet is assumed to contain either C402 (galloxanthin) or C385, and the calculations are repeated, assuming that the UV cone is maximally sensitive at 365 nm rather than 370 nm. The observed match occurred when  $S-$  contained 85–90% 440 nm, which corresponds to the height of the shaded box. The likely range of peak absorbance of the cone oil droplets in the 445 nm cones lies directly under the shaded region on the graph



**Fig. 15** Spectral distribution of the lights used to find a hue match in midspectrum [*narrow, dashed curves*;  $S^-$  (461 nm),  $S^+$  (502 nm),  $S^-$  (520 nm)] and the two participating visual pigments as screened by cone oil droplets (*solid curves* P445 and P508). The spectra of single cones absorbing at longer and shorter wavelengths are shown for comparison (*dotted curves*, P370 and P565). Double cones are also sensitive in this spectral region (*solid curve* P565, calculated as behind pale oil droplets), but as described in [Discussion](#) section, they do not contribute to hue discrimination

and shorter wavelengths, the dip is broad, shallow or indistinct. Second, the logistic regression of  $f$  on  $\log(I_{S^+}/I_{S^-})$  shows a feature not seen at either longer or shorter wavelengths. For most of the mixes of 461 and 520 nm present at  $S^-$ , the curves are statistically flat; however, when 461 nm comprised 0.2–0.4 of the mix, the regressions had a statistically significant *negative* slope (Fig 17). To put it another way, the brighter  $S^+$  was relative to  $S^-$ , the better the birds' performance in

judging a hue match. This can be seen in Fig. 18 by the increasingly pronounced minimum in the hue discrimination function as the relative intensity of  $S^+$  increased. These results are summarized in Fig. 19, where the solid curve represents the aggregated data across all birds and values of  $\log(I_{S^+}/I_{S^-})$  and the dashed curve is for  $\log(I_{S^+}/I_{S^-}) = 0.2$ . In both cases there is a clear minimum at  $S^- \equiv (30\% \text{ 461 nm} + 70\% \text{ 520 nm})$ , although the dip is broad. The effect of intensity will be treated further in [Discussion](#) section.

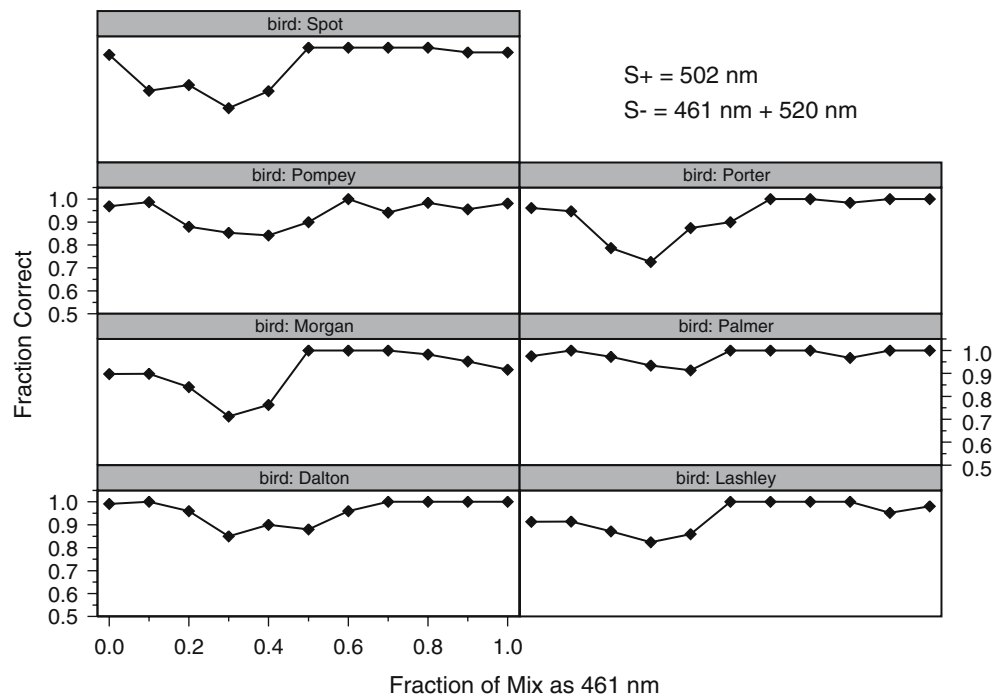
Figure 20 shows how predicted matches vary with properties of the cone oil droplets associated with P445 and P508. The observed hue match (horizontal arrow) is consistent with a high carotenoid absorbance in the yellow droplets in the P508 cones (Goldsmith et al. 1984) and absorbance of galloxanthin in excess of 2.5 in front of P445, as found in the short-wavelength hue match (Fig. 14).

### Intensity discrimination at long and short wavelengths

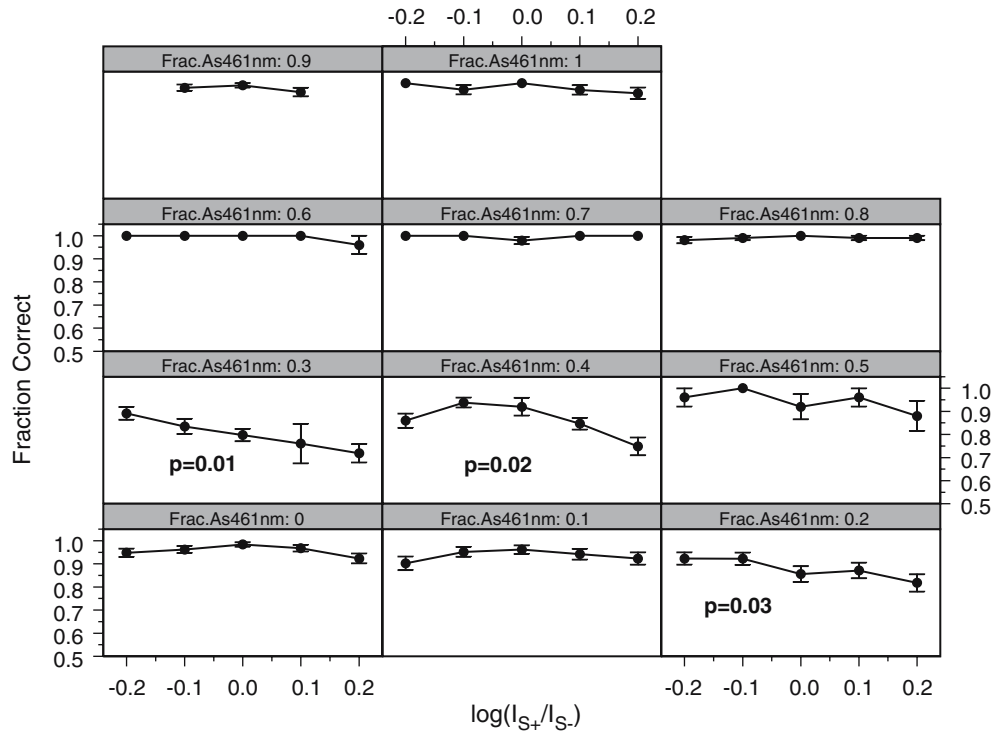
The different effects of intensity at the two ends of the spectrum raise the question whether intensity discrimination might be easier at long than at short wavelengths. This could be the case, for example, if intensity discriminations were mediated solely by double cones. The argument is that double cones all contain the long-wavelength visual pigment ( $\lambda_{\max}$  565 nm), but with absorption at short wavelengths screened to varying extents by cone oil droplets in the primary member and other material in the accessory cone (Fig. 21).

Birds were trained to discriminate a wavelength at fixed intensity ( $S^+$ ) from the same wavelength at a

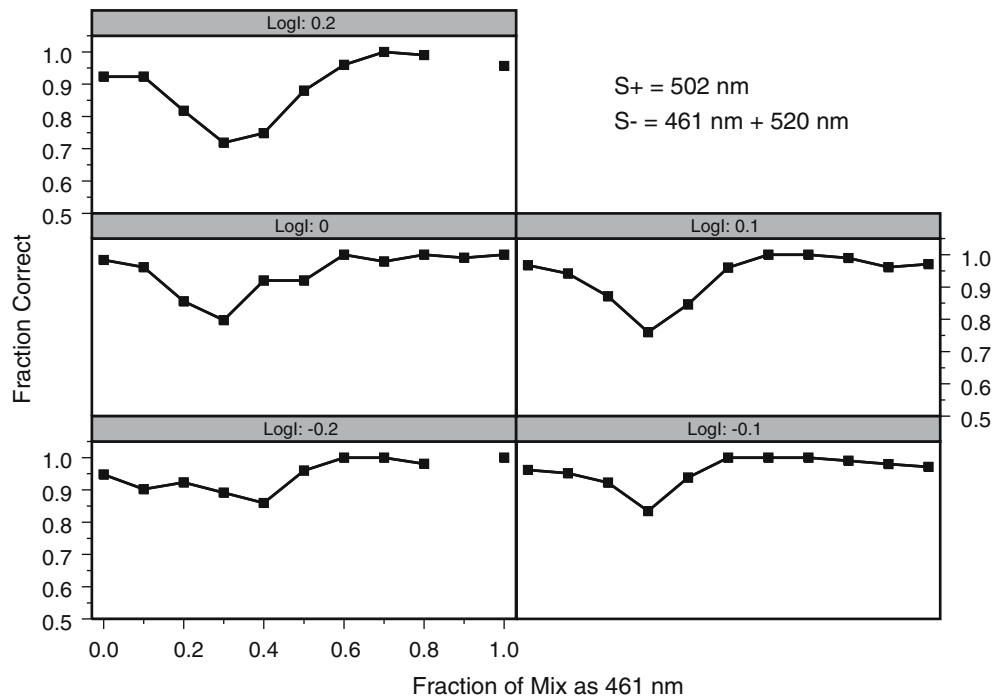
**Fig. 16** Midspectrum hue match, shown for seven individual birds. Several of the birds show a shallow dip in the fraction correct around 30% 461 nm. Data averaged across all intensities



**Fig. 17** Same experiment as in Fig. 16; fraction correct as a function of  $\log(I_{S+}/I_{S-})$  for 11 mixtures of (461 + 520 nm) present at  $S-$ . For 20–40% 461 nm, the logistic regression of fraction correct on the log of the intensity ratio had a statistically significant negative slope. Data averaged over all birds



**Fig. 18** Same experiment as in the preceding two figures, showing that a putative hue match at 30% 461 nm becomes more conspicuous as  $\log(I_{S+}/I_{S-})$  increases. Data averaged over all birds

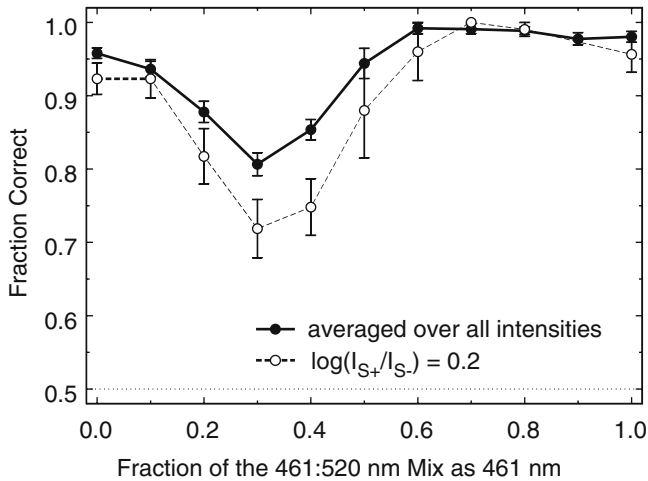


lower intensity at  $S-$ . In a series of tests,  $I_{S-}$  was increased quasirandomly, presenting the birds with decreasing ratios of  $I_{S+}/I_{S-}$  until their ability to discriminate the two targets had deteriorated. Initial training and testing was done at 600 nm. Subsequent testing, first at 420 nm, then 600 nm, showed no difference at these two wavelengths (Fig. 22). When considered alone, the two data points at 0.1 on the energy axis

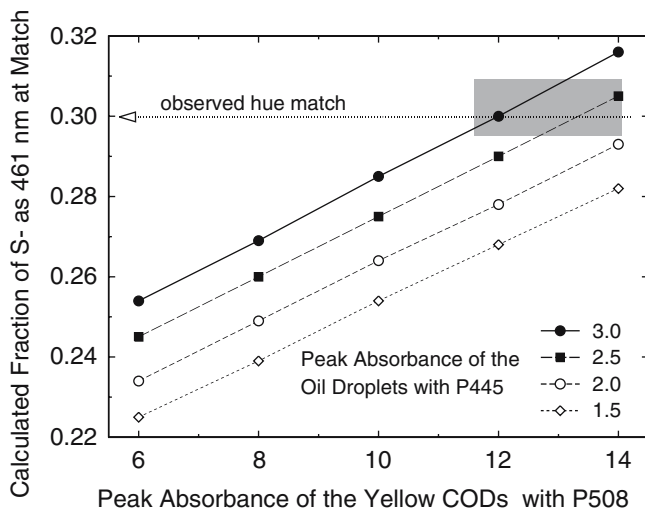
are not significantly different. This experiment will be considered further in the Discussion section.

#### Variation among birds

Each bird's aggregate fraction-correct was calculated for each of the five experiments on color discrimination.

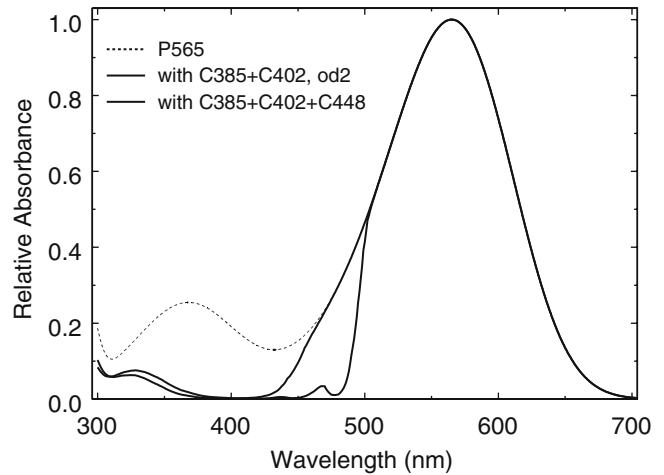


**Fig. 19** Experiment of Figs. 16, 17 and 18 showing that a hue match at 30% 460 nm is clear when the data for all seven birds and the five intensity ratios are pooled (*filled circles*,  $\pm 1$  SE). The minimum deepens when only the data at the highest value of  $\log(I_{S+}/I_{S-})$  are used (*open circles*,  $\pm 1$  SE). The deeper minimum is comparable to that in the long-wavelength match ( $f = 0.7$ ), but the match is not nearly as sharp as at either longer or shorter wavelengths



**Fig. 20** Calculated hue match of 502 nm  $\equiv$  (30% 461 nm + 70% 520 nm) as a function of the peak absorbance of the yellow oil droplets in the cones containing P508 nm. The four curves are for different peak absorbances of galloxanthin (C402) in the oil droplets of the cones containing P445. The *horizontal dotted arrow* indicates the match point found experimentally (Fig. 19). The *shaded box* indicates the region of hue match and oil droplet absorbances that are reasonably consistent with the experimental findings

Table 2 shows the rank order for the six birds that participated in all of these tests. No single bird stands out as invariably better than all the others; for example, four different birds ranked first in the five separate experiments, and rank could vary with subsets of data within an experiment. On the other hand, two of the birds consistently ranked near the bottom. Most of the



**Fig. 21** Calculated effects of cone oil droplets on the absorbance of the visual pigment (P565) in the principal member of double cones. *Dashed curve*, P565 unfiltered. The colorless droplets present in some cones have oil droplets containing C402 (galloxanthin), or C385, or a mixture of these two. The screening effect of a 60:40 mixture with a peak absorbance (od) of 2 is shown by the *left, solid curve*. Pale yellow or greenish droplets also contain a carotenoid (C448), whose addition further depresses sensitivity to wavelengths in the blue, as shown by the *right, solid curve*. The mixture of carotenoids and the maximum absorbance of the droplet vary considerably, even in the same retina (Goldsmith et al. 1984)

differences among birds seem to relate to personality and to daily fluctuations in motivation.

We encountered a visual defect in only one instance. Dalton, who consistently ranked in the top half of the group, had genuine difficulties with the short-wavelength hue match. Based on Experiments 1 and 2, it appears to have had functional UV cones. This bird was an albino with pink eyes and completely white feathers. The absence of carotenoids in the feathers suggested she might also have had a defect in the pigmentation of the cone oil droplets, which could have made her long-wavelength single cones sensitive at short wavelengths. She died of natural causes after the completion of the experimental work, allowing further examination of the eye. There was no melanin in the pigment epithelium, but the retina appeared to contain a normal complement of colored oil droplets. The possibility of abnormal absorption in the colorless droplets that absorb at short wavelengths cannot be eliminated by this observation, but the absence of a normal pigment epithelium with attendant scattered light within the retina may provide an adequate explanation for her difficulty with the short-wavelength hue matches.

## Discussion

The problem presented by double cones

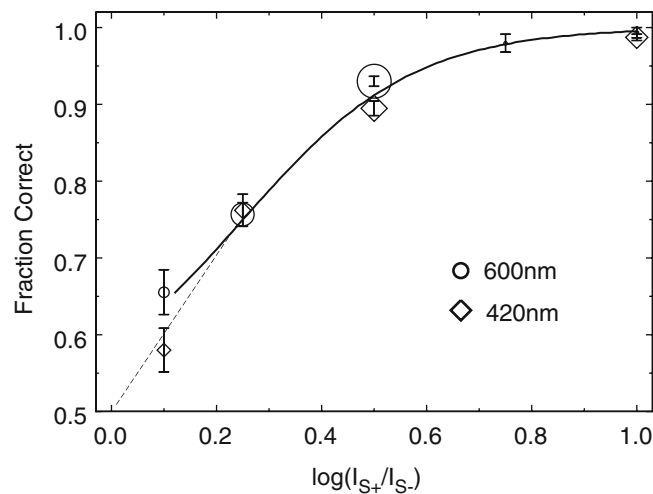
Although the double cones comprise roughly half of the cone population, their role in avian vision is not well understood. In some species, their oil droplets can vary

**Table 2** Rank order of fraction correct by experiment for each of the six birds that participated in all of the hue discrimination tests

Bird	Expt. 1 UV/yellow	Expt. 2 UV/blue	Expt. 3 Yv(R + G)	Expt. 4 Vv(UV + B)	Expt. 5 BGv(B + G)	Average rank
Porter	2	1	3	2	4	2.4
Dalton	1	3	1	6	2	2.6
Palmer	3	5	2	3	1	2.8
Spot	4	4	4	1	5	3.6
Lashley	5	2	6	5	4	4.4
Morgan	6	6	5	4	6	5.4

from colorless in the dorsal retina (where they contain only galloxanthin and/or a UV-absorbing carotenoid) to pale yellow or greenish in the ventral retina (where they contain a mixture of carotenoids). Furthermore, the proportions in the mixture can vary in the same retina (Goldsmith et al. 1984). These features seem poorly fitted for a role in color vision. Evidence exists for their involvement in movement detection, brightness discrimination, and spatial pattern recognition (Sun and Frost 1997; Campenhausen and Kirschfeld 1998; Osorio et al. 1999a). There are also experiments suggesting that they have little or no role in color vision (Osorio et al. 1999b; Smith et al. 2002; Jones and Osorio 2004).

Color vision theory is firmly rooted in human data, where the sensations of hue, saturation, and brightness arise from both antagonistic and convergent interplay of three spectral classes of cone (e.g., Hurvich 1981). Not having double cones, human sensory experience therefore provides no compelling insight about an achromatic channel that is driven by double cones whose sensitivity does not cover the entire spectrum and varies in different retinal regions or how signals from double cones might interact with the perception of color mediated by the single cones. With this preamble, we turn to the present results.



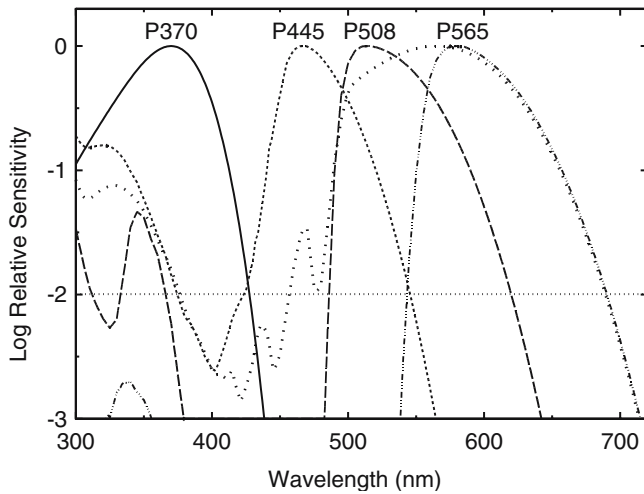
**Fig. 22** Discriminating differences in intensity was equally good at 420 nm (diamonds) and 600 nm (circles). Error bars are  $\pm 1$  SE. Area of each symbol is approximately proportional to the number of observations at that point. Solid curve is the logistic regression of  $f$  on  $\log(I_{S+}/I_{S-})$  for the aggregated data; the dashed line is a linear extrapolation

Hue matches, opponent processes, and the influence of intensity

In overview, Fig. 23 shows calculations of log sensitivity of the four classes of single cones, each filtered by cone oil droplets in ways consistent with the results of the present work as well as previous findings. Except below about 370–380, where the involvement of the 385 nm carotenoid has not been adequately studied and further filtering may well exist, the cone oil droplets are very effective long-pass filters. Second, the budgerigar's threshold detection of hue (Goldsmith and Butler 2003) is consistent with a model (Vorobyev and Osorio 1998) that is based on receptors providing pair-wise input to opponent neural processors in a manner consistent with both Fig. 23 and the hue matches reported in the present work. Fig. 24 summarizes a minimal model for the budgerigar's color vision that is consistent with the results of the hue matches reported here. The system is tetrachromatic in the sense that four cone pigments participate and all are stimulated by sunlight. Of the six possible opponent systems involving pairs of visual pigments, the present work indicates that at least the three involving spectrally adjacent pigment pairs are present. Data do not yet exist to show that for birds, the presence of UV and red wavelengths generates non-spectral colors. P565/P508 and P445/P508 opponent pairs have relatively broad spectral overlap, but P370/P445 much less so (Fig. 23), suggesting particularly good wavelength discrimination in the violet region of the spectrum. P445/P565 opponency (if it exists) would have comparably little spectral overlap. There is no significant spectral overlap of P370 with either P508 or P565.

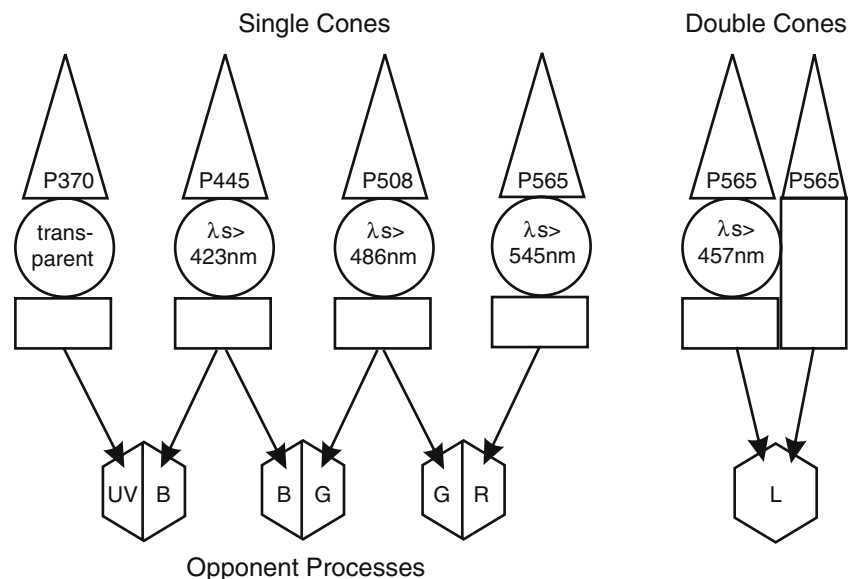
The situation is undoubtedly more complex because two receptors can have convergent input to one side of an opponent process, as the red and green cones together provide the yellow input to the B/Y opponent process in Old World primates. Evidence for the involvement of UVS cones, either alone or summing with other cones as one input to opponent processes, has been found in retinal neurons of turtles (Ventura et al. 2001).

When this work was started there was no experimental basis for equalizing brightness differences for the budgerigar, nor information on how a brightness signal from double cones might make its presence known. The experiments were therefore designed to elicit hue discriminations in the face of variations in  $I_{S+}$ , and this



**Fig. 23** Calculated spectra of log sensitivity for the four visual pigments of single cones, assuming filtering by cone oil droplets with absorbances consistent with the hue matches reported in this paper. Sensitivity of double cones (containing P565) is shown by the *dotted curve* with the broad spectrum extending to short wavelengths. The *horizontal dotted line* indicates 1% of peak sensitivity. Only in the UV at wavelengths shorter than about 380 nm are there possible leaks exceeding 1%, and these may be over-stated, as there is insufficient information about the distribution of the oil droplet carotenoid with  $\lambda_{\text{max}}$  at 385 nm

**Fig. 24** A minimal model for the color vision system of the budgerigar that can be inferred from this and earlier work. The four single cones appear to feed at least three opponent processes, as indicated by the hexagons. The 1% cut-off wavelengths for the long-pass filtering effects of the cone oil droplets are indicated by the numbers in the circles, although the value for double cones is known to vary, depending on the composition of carotenoids in the droplet. The accessory member of the double cones does not have an oil droplet, but there is probably some short-wavelength filtering in the inner segment of the cone. The present results are consistent with the hypothesis that signals from double cones can be seen as



way of framing the birds' task may have reduced the potential salience of brightness. What remains in this discussion is to consider how the observed effects of intensity can be explained and to examine the possible role of double cones.

#### *Hue match at short wavelengths*

In the present work, hue discrimination at short wavelengths—where the sensitivity of double cones should be very low—is accounted for quantitatively by a pair of single cones containing P370 and P445. For three of the birds, performance fell to chance at match. The fact that average correct choices closely approached chance near the match (Fig. 12) suggests that we had actually hit the exact proportions in  $S^-$ , the match might have been complete, i.e.,  $S^+$  and  $S^-$  might have appeared identical. This outcome is potentially possible because red and yellow cone oil droplets block short wavelengths from reaching P565 and P508, and consequently P445 and P370 are the only cone pigments active in this spectral region. Further, unlike the results at longer wavelengths, varying the relative intensities of  $S^+$  and  $S^-$  around the match did not change the frequency of correct choices.

#### *Hue match at long wavelengths*

In the long wavelength region of the spectrum, a dichromatic hue match was also quantitatively consistent with the absorption properties of two visual pigments, P565 and P508. Unlike the match at the short wavelength end of the spectrum, however, there was a small but significant effect of intensity for difficult discriminations around the match. Double cones are active in the spectral region of this experiment, and if the long-wavelength input to the opponent neural processor had come exclusively from P565 unscreened by red oil droplets (as in double cones), the mixture at match

would have required 0.90 rather than 0.915 red. To put it another way,  $S+$  (590) and  $S-$  (639) excited the double cones in virtually the same ratio as the single cones containing P565. Although double cones could therefore be engaged in this color discrimination, there is an alternative way of looking at the data.

Suppose double cones are not involved in color vision, but that in this experiment they were generating a veiling signal that influenced the birds' choices at spectral mixtures that were close to a hue match. For equal light intensities at  $S+$  and  $S-$  and for the known proportions of 563 and 639 nm in  $S-$  at match, we can generate values of  $\bar{\alpha}_{S,P}$  (Methods) to compute the relative activation of double cones at  $S+$  and  $S-$ . At match, the ratio ( $S+/S-$ ) is 2.48, i.e., double cones are excited more by  $S+$  than  $S-$ . (This can be appreciated qualitatively by the following observation: the high sensitivity of P565 (unscreened in double cones) to  $S-$  (563) (Fig. 6) is discounted in  $S-$  because at match 563 nm contributes only 8.5% of the quantum flux in the mix.) In other words, the addition of this veiling signal adds more to  $S+$  than  $S-$  by about 0.39 log units of intensity. When hue discrimination was easy, differences in the excitation of double cones were ignored. When discrimination became difficult near a match, however, the birds began to attend to the (brightness?) difference generated by the double cones. As the stronger signal from double cones correlated with  $S+$ , the birds' success remained significantly above chance. For the same reason, when  $I_{S+}/I_{S-}$  was increased, the birds' scores bettered further. Thus in this experiment, if a difference in hue was not obvious, selecting the window that generated the stronger signal from double cones tended to provide a reward. Fortunately, the salience of this signal remained low as long as the differences in hue were readily discerned. At match,  $S+$  could have differed from  $S-$  in saturation (if there is a "whiteness" signal originating from single cones), lifting  $f$  slightly above chance, but this effect would be confounded with the postulated contribution of double cones (presumed, for the sake of argument, to provide a discrete sensation).

#### *Discriminating UV in the presence of blue or yellow*

The interpretation that intensity differences can become salient when hue discriminations are difficult is supported by the experiment where  $S+$  and  $S-$  were both 590 nm (Fig. 4). With no UV present, each stimulus excited the same complement of double cones, single cones containing P565, and single cones containing P508. In this control,  $f$  rose above chance when  $S+$  was brighter than  $S-$  and fell below chance when  $S-$  was the brighter window. This effect was not so easily produced when the task was to discriminate either 440 or 420 nm from mixtures of UV and 440 nm, which further suggest that the salient brightness signals likely arose in double cones.

#### Hue match in midspectrum

The hue match in midspectrum was not as sharp as the matches at either shorter or longer wavelengths (Fig. 19), although, based on the sensitivity of single cones in this spectral region (Fig. 15), one might have anticipated a comparable outcome. Second, increasing the intensity of  $S+$  relative to  $S-$  caused the errors around the match point to increase rather than decrease (Figs. 18, 19). Brightness signals from double cones provide no help in accounting for this result. First, the ratio of double cone excitations at  $S+$  to  $S-$  was nearly 1 at match (0.85–0.93, depending on reasonable assumptions about oil droplets screening the principal member of the double cones). The birds were therefore not attracted to a brighter stimulus caused by a greater excitation of double cones; they behaved as though they were still trying to solve the problem of hue discrimination.

The experimental result, however, is consistent with the following explanation. Although only two of the single cones are active in this spectral region (Fig. 15), P508 has input to both a red/green opponent process and a blue/green opponent system that engages P445 (Fig. 24). At the predicted match of 502 nm  $\equiv$  (30% 461 nm + 70% 520 nm), excitation of P508 was the same at  $S+$  and  $S-$ . Increasing the intensity of  $S+$  changed the brightness of  $S+$  in the hue match—and perhaps hue as well, if there is a Bezold-Brücke effect as reported for the pigeon (Wright 1976)—but in addition to a larger signal from double cones, it would also have increased the strength of a chromatic signal generated by 520 nm stimulation of the short-wavelength side of the red/green opponent system. This left the hue at  $S-$  closer in appearance to the original training light, but when birds now selected  $S-$  they were scored as having made an error. Consequently, the dip in the plot of frequency correct v mix (Fig. 19) deepened from 0.8 to 0.7.

This interpretation opens the question of why hue matches were possible at both longer and shorter wavelengths without the intrusion of flanking opponent processes. The likely reason is that in the match of 420 nm  $\equiv$  (10–15% UV + 90–85% 440 nm),  $S+$  produced very little excitation of P445. Similarly, for the match of 590 nm  $\equiv$  (10% 563 nm + 90% 639 nm),  $S+$  provided only modest excitation of P508, and at calculated match, excitation of P508 accounted for only 8.5% of the spectral mixture in  $S-$ . Thus, around the match point at these long wavelengths, increased excitation of the double cones by  $S+$  produced the more significant confounding effect for the birds.

#### Comparison with the pigeon

The most extensive psychophysical work on avian color vision has been done on the pigeon (*Columba livia*). Wright and Cumming (1971) measured color-naming



functions, whose boundaries correspond to regions of good hue discrimination (Blough 1972; Wright 1972b; Emmerton and Delius 1980; Palacios et al. 1990). The only published work on hue matches by birds was also done on pigeons, but pigeons and budgerigars differ in a couple of important ways. One quadrant of the pigeon's retina is particularly rich in red and orange cone oil droplets, and it differs in spectral sensitivity from the remainder of the retina (e.g., Romeskie and Yager 1976; summarized in Hart 2001). Second, rather than the UVS variant, species including pigeons and gallinaceous birds have a VS cone ( $\lambda_{\max}$  near 410 nm in the pigeon) (Govardovskii and Zueva 1977; Fager and Fager 1981; Wortel et al. 1987; Yoshizawa and Fukada 1993; Vos Hzn et al. 1994; Bowmaker et al. 1997; Yokoyama et al. 1998; reviewed in Hart 2001) likely setting the short-wavelength end of the visible spectrum. UV sensitivity of pigeons (Wright 1972a; Kreithen and Eisner 1978), domestic chicks (Osorio et al. 1999b), and quail (Smith et al. 2002) is reasonably attributable to the short-wavelength limb of the VS receptor.

Palacios et al. (1990) showed that at long wavelengths  $S+ \equiv 590$  or  $600$  nm could be matched by  $S-$  mixtures ( $580 + 640$  nm). They concluded that two cones could account for the result, although the data were consistent with the activity of a third (double) cone. This finding and its interpretation are in qualitative agreement with the present work. Palacios and Varela (1992) extended the study to shorter wavelengths. The most interesting comparison with the present data involves their effort to match 400 nm with ( $370 + 450$  nm), an experiment very similar to our match of 420 nm with a mix of ( $365 + 440$  nm). Unlike the budgerigar, which displayed a sharp match in this spectral region, the pigeon found the task difficult and exhibited confusion throughout the range of mixes. This is understandable if the pigeon has only P412 active in this spectral region because P460 has  $\lambda_{\max}$  shifted to about 480 nm (Bowmaker 1977) with steep attenuation on the short-wavelength side caused by absorption in the cone oil droplet. In midspectrum, 470–560 nm, the pigeon was understandably not dichromatic (Palacios and Varela 1992).

### Intensity discrimination

In the present work, the observation that intensity differences seemed to have a greater salience in difficult hue discriminations at long wavelengths (Figs. 4, 9) than at short wavelengths (Fig. 13) suggested that double cones might be involved. If intensity discrimination were the exclusive province of double cones, then the birds might be expected to be more sensitive at this task at long than at short wavelengths. The experiment of Fig. 22, however, failed to show any such difference.

How is this experiment consistent with the different salience of intensity at long and short wavelengths seen in hue discriminations? Hue discrimination clearly engages the single cones. Similarly, if double cones

ordinarily mediate intensity discriminations, their intrusion into difficult hue discriminations may simply be more likely at wavelengths where they are most sensitive. In fact, we observed only one instance in which intensity differences influenced hue discrimination at short wavelengths (Fig. 5, middle panel), and that came at the end of a substantial period of testing, thus after extended experience with a difficult task. But if the birds are *trained* to heed intensity differences, intensity discrimination appears to be good at short wavelengths, where double cones are at a disadvantage. In other words, if the context changes, the salience of intensity in the afferent pathways of single cones may also change. The extent to which double cones are ordinarily responsible for intensity discrimination thus remains an open question, for it is hard to account for the experiment of Fig. 22 by invoking them alone.

In the pigeon the threshold for brightness discrimination in successive contrasts is about 0.11 log unit; in simultaneous contrast it is estimated to be about 0.01 log unit (Hodos 1993). The present experiment involves simultaneous contrast, but the spatial separation of  $S+$  and  $S-$  was too large to measure the birds' best capabilities. With a criterion of 75% correct, sensitivity to intensity differences was about 0.25 log unit, and with a criterion of 60% it was (by extrapolation) about 0.1 log unit. A more critical test of the wavelength dependence of intensity discrimination, using apparatus designed for the purpose, might reveal a larger role for double cones closer to threshold.

### Cone oil droplets

The results of the mid- and short-wavelength matches point to the need for more data on the carotenoid composition and spectral absorbance of the cone oil droplets. Microspectrophotometry of native droplets does not provide an accurate measure of absorption because the measurements are contaminated by scattered light (Liebman and Granda 1975; Varela et al. 1993). Characterizing droplets by half-maximal absorption provides inadequate information about leakage at short wavelengths, and, particularly for red and yellow droplets with very high absorbance, the analyses tend to underestimate the steepness of the short wavelength cutoff. The only data on expanded droplets from bird retinas (Goldsmith et al. 1984) are from small numbers of individual birds representing a variety of species and show considerable variation in absolute absorbance. This variation is likely to have little consequence at long wavelengths, but the results of the present study indicate that consistent hue matches for mid- and short-wavelength matches should be sensitive to the absolute absorbance of the oil droplets screening P445 and P508.

The all *trans* isomers of familiar carotenoids (e.g., lutein, astaxanthin) have little absorption in the near UV, so even when present in moderate concentration

they pass significant light in the spectral region of the UV-sensitive cones. Some of this leakage in yellow droplets is reduced by the presence of the 15, 15' *cis* isomer of xanthophyll (T.H. Goldsmith unpublished observations; spectra in Goldsmith and Butler 2003), which has an absorption band in the near UV. Leakage is also reduced by a carotenoid with  $\lambda_{\max}$  at 385 nm (Goldsmith et al. 1984), but there is little information on the distribution of this molecule. It and/or galloxanthin are the likely sources of the weak fluorescence that can be seen in the more weakly pigmented oil droplets.

### Why UV?

UV receptors are present in many insects and vertebrates. Most mammals lack UV receptors, reflecting a long evolutionary period of nocturnality during which they lost two cone pigments and the cone oil droplets (Goldsmith 1990). To be more precise, contemporary mammals have cone pigments in the two evolutionary lineages represented by the LWS and UVS/VS pigments of birds. In most mammals the UVS/VS pigment is maximally sensitive in the violet (Yokoyama and Shi 2000). In Old World primates, a relatively recent gene duplication has created two spectral variants of the LWS pigment, allowing trichromacy (Nathans et al. 1986).

UV cone pigments extend the visible spectrum by 50–70 nm and enrich color vision (Vorobyev 2003). Relatively few UV cones suffice; compared to the cones maximally sensitive at longer wavelengths, they are relatively little adapted by ambient sunlight (Goldsmith and Butler 2003) and therefore make a substantial contribution to the measure of photopic sensitivity that is mediated by single cones and is revealed behaviorally by tests of color discrimination. UV signals have been exploited where they occur. Birds may use UV cues in foraging and do use UV in reflectance spectra of feathers in mate choice (reviewed in Cuthill et al. 2000). There is no evidence in birds for hard-wired, wavelength-dependent behaviors that exist in parallel with color vision (Goldsmith 1994) as exist in honeybees. Sexual selection (as in species-specific behavior involving mate choice) or individual experience (as in selection of flowers by hummingbirds) may endow particular colors with context-specific salience, but the perceptual processes employ general-purpose color vision. The “special” nature of UV signals arise from the relatively modest number of natural sources of UV cues, chief among which (for birds) are structural colors of some feathers. Not only does this signal provide opportunity for color contrast in sexual displays (Hausmann et al. 2003), but also in principle, feedback can enhance sexual selection of feathers as signals of fitness (Keyser and Hill 1999, 2000).

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