Comparison of categorical color perception in two Estrildid finches

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ABSTRACT

Sensory systems are predicted to be adapted to the perception of important stimuli, such as signals used in communication. Prior work has shown that female zebra finches perceive the carotenoid-based orange-red coloration of male beaks—a mate choice signal—categorically. Specifically, females exhibited an increased ability to discriminate between colors from opposite sides of a perceptual category boundary than equally-different colors from the same side of the boundary. The Bengalese finch, an Estrildid finch related to the zebra finch, is black, brown and white, lacking carotenoid coloration. To explore the relationship between categorical color perception and signal use, we tested Bengalese finches using the same orange-red continuum as in zebra finches, and also tested how both species discriminated among colors differing systematically in hue and brightness. Unlike in zebra finches, we found no evidence of categorical perception of an orange-red continuum in Bengalese finches. Instead, we found that the combination of chromatic distance (hue difference) and Michelson contrast (difference in brightness) strongly correlated with color discrimination ability on all tested color pairs in Bengalese finches. The pattern was different in zebra finches: this strong correlation held only when discriminating between colors from different categories, but not when discriminating between colors from within the same category. These experiments suggest that categorical perception is not a universal feature of avian, or even Estrildid finch, vision. Our findings also provide further insights into the mechanism underlying categorical perception and are consistent with the hypothesis that categorical perception is adapted for signal perception.

Keywords: Zebra finch, Bengalese finch, color signaling, avian vision, carotenoid, sensory ecology

Introduction

To efficiently and accurately assess signals, animals filter out irrelevant information, or simplify or enhance relevant variation, using a variety of perceptual mechanisms (Wehner 1987). One such mechanism is categorical perception, in which an animal's perceptual system sorts continuously-varying stimuli into discrete categories (Harnad 1987). With categorical perception, individuals sort, or "label," continuous variation along some dimension of a stimulus into categories. Individuals also exhibit enhanced discrimination of stimuli from different sides of the category boundary as compared to equally different stimuli from within the same category. Although the stimulus varies continuously, certain equally-distinct variants are perceived as more distinct from one another depending on whether they are located on the same side or different sides of a category boundary. The extent to which categorical perception functions in signal assessment has important implications for our understanding of the selection pressures shaping both perceptual processes and signal form (Green et al. 2020).

Originally described for human speech (Liberman et al. 1957), categorical perception has since been demonstrated in signaling contexts across a variety of taxa and sensory modalities (reviewed in Green et al. 2020). Recently, female zebra finches (*Taeniopygia guttata*, family Estrildidae) were shown to categorically perceive an orange-red color range that corresponds to variation in the carotenoid-based coloration of male zebra finch beaks (Caves et al. 2018), a signal that females assess during mate choice (Burley and Coopersmith 1987; de Kogel and Prijs 1996; Blount et al. 2003). Female zebra finches also categorically perceive a blue-green color range that has no known signaling function in this species (Zipple et al. 2019), but key differences exist between the structure of blue-green and orange-red categories. In particular, females exhibit greater within-category discrimination in the blue-green compared to the orange-red. One potential explanation for these differences is that selection has acted on how the visual system perceives orange-red coloration given that it plays an important signaling role. Comparative studies, particularly between species with similar visual physiologies but different signaling

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traits, provide a powerful tool by which to examine the selective influence of signal perception on perceptual processing and how receivers may coevolve with signalers (Price 2017).

Here, we test for categorical perception of carotenoid-based coloration in another Estrildid finch, the Bengalese finch (*Lonchura striata domestica*). Domesticated primarily from the white-rumped munia (*Lonchura striata*) within the last 250 years (Colquitt et al. 2018), Bengalese finches display characteristic piebald coloration, with birds ranging from nearly all white to nearly all black (Eisner 1960). Bengalese finches and their wild ancestor are sexually monomorphic, and aside from black, brown, and white, display no obvious coloration, including carotenoid-based coloration. This lack of a carotenoid-based signal makes them a good species in which to examine if and how perceptual systems are matched to signal expression.

Categorical perception makes clear predictions regarding labeling and discrimination of stimuli (Studdert-Kennedy et al. 1970). To demonstrate labeling, one tests individuals' abilities to discriminate between variants when comparing one endpoint of a stimulus continuum to variants occurring at increasing distances along that continuum. Categorical perception predicts that at a certain point along the continuum—the putative category boundary—individuals change from labeling two stimuli as "similar" to labeling them as "different," which in non-human animal studies is represented by an abrupt increase in the ability to tell two stimuli apart. Importantly, this putative boundary should be found at the same point on the continuum regardless of which endpoint the test stimulus is being compared to. Labeling tests thus allow one to generate a hypothesis regarding the location of a category boundary. The second requirement is a significant increase in the ability to discriminate between two stimuli from opposite sides of the hypothesized boundary, relative to two equally-different stimuli drawn from the same side of the boundary.

We used behavioral assays to test for labeling and discrimination in Bengalese finches along the same orange-red carotenoid color continuum previously tested in zebra finches (Caves et al. 2018).

Counter to what has been found in zebra finches, our labeling trials did not show evidence of a category boundary, and discrimination trials pointed to differences in brightness between two colors as a better predictor of discrimination ability in Bengalese finches than a category boundary. Therefore, we further assayed color discrimination ability in both species using an additional set of colors that provided us with data on discrimination ability for color pairs that differed systematically in both hue (i.e. how red or orange they were) and brightness. These additional color discrimination assays provided data for predictive models that allowed us to examine the influence of hue, brightness, and (if present) categorical perception on color discrimination in each species.

Methods

Experimental Subjects

Subjects were sexually mature female Bengalese and zebra finches. Zebra finches were obtained from a colony maintained by Richard Mooney at Duke University (IACUC A258-14-10). Bengalese finches were obtained from Magnolia Bird Farm (Riverside, California, USA). Female Bengalese finches were identified using PCR following the methods described in Griffiths et al (1998).

Birds were housed in individual cages (12 x 18 x 13 cm, Prevue Pet) outfitted with perches, a cuttlebone, and *ad libitum* water and seed (Kaytee Forti-Diet Pro Health Finch diet). Outside of trials, lighting was provided by fluorescent bulbs (Ecolux with Starcoat SP 35/41, color temperature 3500-4100K, General Electric) with ballast (Hi-Lume 3D/Eco-10, Lutron Electronics) operating at 60Hz and kept on a 15h:9h light:dark cycle. Rooms were maintained at 25-27 °C. All methods were approved under Duke University IACUC protocol A004-17-01.

Color stimuli

To assess color discrimination ability, we followed methods described in Caves et al. (2018). In brief, previous work showed that the beak colors of male zebra finches can be approximately represented by red and orange colors in the Munsell color system. Thus, we used as a starting point a set of 40 Munsell colors previously identified as capturing the variation in male zebra finch beak coloration (Burley and Coopersmith 1987; Collins et al. 1994; Birkhead et al. 1998). We measured reflectance spectra from each of these 40 colors (Munsell color paper, Pantone LLC, Carlstadt NJ, USA) using an integrating sphere with a built-in tungsten-halogen light source (ISP-REF; Ocean Optics), relative to a Labsphere Spectralon 99% white standard.

For each measured color, we calculated photon catches, which quantify the relative stimulation of each photoreceptor type in response to viewing a certain stimulus. In contrast to primates, birds are thought to have separate sets of photoreceptors for encoding brightness information (double cones) and color information (single cones) (Osorio and Vorobyev 2005). Thus, we quantified relative photon catches for both the double and single (ultraviolet-, short-, medium-, and long-wavelength) cones as measures of perceived brightness and color, respectively. Relative photon catches were calculated over wavelengths from 400-700nm (Table S1), using the following formula:

$$Q_{r,c}(\lambda) \propto \int_{400}^{700} S_r(\lambda) * R_c(\lambda) * I(\lambda) \, d\lambda$$

in which Q is the photon catch for photoreceptor type r in response to color c, S_r is the spectral sensitivity (i.e., sensitivity to light of different wavelengths) in zebra finches of photoreceptor type r (data from Lind 2016), R_c is the reflectance of color c, λ denotes wavelength, and I is the irradiance of the ambient illuminant. As an ambient light spectrum, we used the CIE Illuminant A standard tungsten bulb illuminance spectrum (color temperature 2856K). Illuminant A is nearly identical to the actual

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ambient light in our experimental room (Figure S1), and predicted color discriminability under standard and experimental lighting conditions were nearly identical (Table S2).

We used the log of the photon catch values to calculate measures of the brightness and hue differences between color pairs. As a measure of the brightness difference between two colors, we calculated the Michelson contrast (Cronin et al. 2014), which is the ratio of the difference to the sum of the double cone quantum catches (Q_1- Q_2 / Q_1+Q_2). For hue difference, we calculated chromatic distance (ΔS, a measure of the predicted discriminability between two colors). ΔS was calculated using the receptor noise-limited (RNL) model of color discrimination (Vorobyev and Osorio 1998), which assumes that the ability to discriminate two colors is limited by noise in the photoreceptors. We then visualized ΔS in a perceptually uniform, two-dimensional space based on hue and saturation (also known as chroma, or color intensity) in which the Euclidean distance between two colors is equivalent to the RNL model-derived chromatic distance (equations for chromaticity space in Hempel de Ibarra et al. 2001).

This chromaticity space can be used for trichromatic vision (visual systems in which three cone types convey color information). Although birds have four cone types that contribute to color vision, the use of trichromatic vision under our particular experimental conditions is appropriate because, under our experimental lighting, the reflected ultraviolet radiance, and thus the contribution to color vision from the ultraviolet (UV) cone is essentially zero (Figure S1). Thus, the quantum catch for the UV cone was on average (± standard deviation) only 0.26 ± 0.10 % (range 0.14% - 0.42%) of total single cone quantum catch (see Table S1). Recalculating ΔS using a tetrachromatic visual system (i.e., including the UV cone catch) had minimal impact on predicted discriminability (Table S2), changing ΔS values by a mean (± standard deviation) of 0.26 ± 0.41 (range -0.18 - 0.99). Therefore, we expect that the impact of the UV cone on color perception was minimal, and did not include the UV photon catch in our calculations.

Assuming a trichromatic visual system also allowed us to visualize the relative positions of all 40 Munsell color swatches that we measured by their X and Y coordinates in the chromaticity space described above, in which Euclidean distance is equivalent to ΔS (Hempel de Ibarra et al. 2001). We used those coordinates to select two sets of colors for use in behavioral experiments (Figure 1). The first set (the 'Beak Set') was a set of eight colors, previously used in Caves et al. (2018), chosen because those colors (1) span the full range of colors previously used to describe male zebra finch beaks (Burley and Coopersmith 1987; Collins et al. 1994; Birkhead et al. 1998) and (2) are approximately equally spaced the chromaticity space described above that is based on zebra finch spectral sensitivity, and thus are predicted to be equally discriminable from one another, based on chromatic cues. As with real beak colors, the colors in the Beak Set also varied in perceived brightness. Female zebra finches categorically perceive the Beak Set, with a boundary between colors 5 and 6 (Caves et al. 2018).

The second set (the 'Extended Set') was chosen to provide further information about the relative effects of chromatic distance and Michelson contrast on discrimination ability. Behavioral color discrimination data from the Extended Set were used as training data for a predictive model of color discrimination ability (see 'Statistical Analyses' for details). To create the Extended Set, we replaced four colors in the Beak Set with new colors, with the resultant Extended Set spanning the same range in color space and in brightness, and having roughly the same chromatic distance between neighboring colors, as the Beak Set (Figure 1). However, rather than steadily increasing in brightness, the extended colors each differed from their neighboring colors by a Munsell value (the measure of brightness in the Munsell system) of one. Colors 5 and 6—which span the previously described location of the category boundary in the Beak Set—were the same in the two color sets.

Throughout, relative photon catches, and thus brightness and ΔS, were calculated using spectral sensitivity curves for the zebra finch (Bowmaker et al. 1997; Lind 2016), because spectral sensitivity curves for Bengalese finches are not available. However, both species have UVS type visual systems (one

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of two types of color vision systems found in birds; Hart 2001), and variance in peak spectral sensitivities of both single and double cones across Estrildid finches is extremely low (Figure S2; Hart et al. 2000), so it is likely that Bengalese and zebra finches have similar spectral sensitivities. Additionally, recalculating ΔS using either a different UVS visual system (the starling, *Sturnus vulgaris*), the average UVS cone-type retina, or the average VS cone-type retina, which is the other primary type of retina found in birds (data from Endler and Mielke 2005), had little impact on relative chromatic distances (Table S2). Thus, ΔS is robust even to large differences in spectral sensitivity for this set of colors and so is unlikely to differ substantially between Bengalese and zebra finches.

Behavioral tests of color discrimination

For both the Beak Set and the Extended Set, we created disc stimuli by gluing together two semi-circular halves of Munsell paper to form a circle. The two halves were either the same color ('solid') or different colors ('bicolor') and covered with a clear epoxy cover. We tested color discrimination using a food-reward protocol in which birds were presented with a foraging grid containing 12 wells, six of which were covered by the disc stimuli described above, two by bicolor discs and four by solid discs (two of each color comprising the bicolor discs). We trained birds to search for food rewards beneath bicolor discs made of the two endpoint colors from the Beak Set, 1 and 8 (i.e., "1|8"). Birds passed a trial if they removed both bicolor discs before any solid discs, which would occur by chance in only 1/15 trials. We gave birds two minutes to pass each trial. Birds that passed six out of seven consecutive training trials began experimental trials.

In experimental trials, the makeup of the discs on the grid was the same as in the training trials, but we varied the two colors comprising the discs. Two types of trials were run: labeling and discrimination. Labeling trials used bicolor discs that included the endpoint colors in combination with all other colors, for example 1|2, 1|3, 1|4, etc. and 8|7, 8|6, 8|5, etc. In discrimination trials, discs

comprised color combinations that were equally spaced across the continuum, meaning they were either one (i.e. 1|2, 2|3, 3|4, etc.), two (i.e. 1|3, 2|4, 3|5, etc.), or color three steps apart (i.e. 1|4, 2|5, 3|6, etc.), referred to below as "one-apart," "two-apart," and "three-apart," respectively.

On experimental trial days, we removed food at 0900, to ensure that birds were motivated to perform the task, and began trials at 1400. During trials, lighting was provided by halogen bulbs (color temperature 2900K, model number H&PC-61361, Philips Lighting) hung approximately 80 cm above the cage and filtered through vellum paper to provide diffuse lighting. Birds were allowed at least five minutes to acclimate to the experimental lighting conditions before trials began.

To assess whether female Bengalese finches perceive carotenoid-based coloration categorically, as zebra finches do (Caves et al. 2018), we first ran labeling and discrimination trials on female Bengalese finches (n=10) using the Beak Set. To further assess the relative contributions of chromatic distance and Michelson contrast to discrimination ability, we performed discrimination trials using the same Bengalese finches (n=10) and the Extended Set of colors. Finally, to compare zebra finch perception with Bengalese finch perception across both color ranges, we performed discrimination trials on female zebra finches (n= 8) using the Extended Set. Data on female zebra finch labeling and discrimination of the Beak Set (n=26) were taken from Caves et al. (2018).

As in Caves et al. (2018), we also performed trials in which the discs were the same color on each half (1|1 or 8|8) to control for the possibility that birds detected seeds by olfaction. In both cases birds performed no better than chance, indicating that they did not use olfactory cues in determining which discs to flip. These olfaction-control comparisons are not included in further analysis. We randomized the location of discs on the grid for each trial using the *sample* function in R (R Development Core Team 2019). For each bird, we performed ten trials for each color combination (all individuals participated in at least five trials for each color combination) and calculated "pass frequency," the proportion of trials that they passed for a given color combination. Throughout, we use pass frequency

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as a measure of color discrimination ability. Each day, trials began with a refresher, in which birds were given one trial with 1|8 discs, to ensure they remembered the trained task, and ended with a motivation check, in which we recorded the amount of time it took birds to begin eating seeds out of their regular dish once it was returned to the cage, to ensure that birds had remained hungry and motivated throughout the task.

Statistical Analyses

Data used for all analyses are available in the Dryad Digital Repository (https://doi.org/10.5061/dryad.stqjq2c1z; Caves et al. 2021). We found no evidence for categorical perception in Bengalese finches (see Results), but Michelson contrast appeared to play an important role in discrimination ability. This finding was different than prior results from zebra finches, in which a color category boundary best explained color discrimination ability. To statistically explore the relative impacts of a color category boundary or Michelson contrast on color discrimination ability, we built linear mixed models (using *lme4*, Bates et al. 2015) to describe the average pass frequency for each type of discrimination task (one-apart, two-apart, or three-apart) for each species. We used *lmerTest* (Kuznetsova et al. 2017) to estimate degrees of freedom and calculate *p* values via Satterthwaite's method. As the response variable, we used the pass frequency for each bird for each color combination. Predictor variables were either a binary indicator of whether a given comparison crossed the 5-6 boundary (the 'category' model), or Michelson contrast between color pairs (the 'contrast' model). For the models that considered one-apart, two-apart, or three-apart data on their own, we did not include chromatic distance as a predictor, because all comparisons within a discrimination set are approximately equally spaced by design. However, we did also build 'category' and 'contrast' models that included all discrimination data (one-apart, two-apart, *and* three-apart) and the predictor of chromatic distance.

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Model fit was assessed using the Akaike Information Criterion (AIC), a value which can be used to determine which of a set of models is most likely to be the best model for a given data set (Burnham and Anderson 2002 and references therein). Change in AIC value (ΔAIC) was calculated relative to the better fitting model in each case, and following Burnham et al. (2011), ΔAIC values were used to calculate relative likelihoods for each model i within a set using the formula

$$l_i = \exp[-(1/2) \, \Delta AIC_i].$$

We then calculated the Akaike weight for each model by dividing the likelihood of a given model i, by the sum of the likelihoods of all models within that set (Burnham et al. 2011). The Akaike weight gives the probability that each model within a set of models is the best model.

To further examine differences between Bengalese finches and zebra finches in their color discrimination abilities, we built a model to predict how discriminable a color pair should be for each species, depending on chromatic distance and Michelson contrast. We built this model using pass frequency data from the Extended Set, because color comparisons in that set had been chosen to vary systematically in both chromatic distance and Michelson contrast (the Extended Set colors were the same for both species, but separate predictive models were built for Bengalese and zebra finches). We then tested how well data from the Beak Set matched the predictive model built from the Extended Set. We specifically wanted to examine how pass frequencies for comparisons that do or do not cross the 5-6 boundary in the Beak Set were predicted by chromatic distance and Michelson contrast (i.e. brightness differences) in both Bengalese finches and zebra finches. Thus, to avoid circularity that would result from building the model using data on cross-boundary comparisons that it would then be used to predict, we built the model using the 13 comparisons from the Extended Set that did not include the original 5-6 category boundary (i.e. no comparisons used in the model included the color step between colors 5 and 6, Table S3).

These predictive models (one for each species) were fixed-intercept linear models that included pass frequency as the response variable, and both chromatic distance and Michelson contrast as predictors. The intercept was set to the probability of a bird passing a task by chance (i.e. 1/15). We found that the predictive models well-described the data used to construct them. In each species, the predictive model that included both terms performed better than did a model that included only chromatic distance or Michelson contrast (Supp Table S5). Specifically, in both species, pass frequency for the Extended Set was strongly predicted by the combination of chromatic distance and Michelson contrast (for Bengalese finches $R^2 = 0.96$; for zebra finches $R^2 = 0.91$) and coefficient estimates were similar in both species (see Table S4).

The coefficients from the predictive model represented expected pass frequency for color combinations of a given chromatic distance and Michelson contrast. We then visualized how observed pass frequency on discrimination tasks from the Beak Set aligned with the expected pass frequency values generated by the model by plotting observed versus expected pass frequency. In this plot, points falling on the line of slope 1 were those in which observed pass frequency was perfectly aligned with expected pass frequency, i.e. perfectly predicted by chromatic distance and Michelson contrast.

Lastly, we built linear models for each species of observed pass frequencies for color comparisons in the Beak Set as predicted by (1) expected pass frequency, (2) whether the Beak Set comparison included the 5-6 category boundary, and (3) the interaction between these terms. When categorical perception is operating, the combination of chromatic distance and Michelson contrast should have different effects on discrimination of comparisons that cross the category boundary as compared to those that do not. We therefore predicted that the interaction term in the model, between expected pass frequency and crossing the 5-6 boundary, should be significant only if a species exhibits categorical perception.

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Evaluating sample size differences between Bengalese finches and zebra finches

To ensure that the apparent lack of categorical perception in Bengalese finches was not simply

due to having a smaller sample size of Bengalese finches than in the original zebra finch study (Caves et

al. 2018), we performed a resampling analysis in which we used linear mixed effects models to calculate

the effect of each color step on pass frequency (see Figure S3 for details). We randomly resampled 10

individuals (the sample size of Bengalese finches) from the original zebra finch data set (n = 26) 1000

times, and for each permutation calculated the effect of each color step on pass frequency. This analysis

showed clearly that, even if the sample size of zebra finches was only 10, the effect of the 5-6 color step

is larger than that of any other color step, as is consistent with categorical perception at the 5-6

boundary, in all but six out of 1000 permutations (Figure S3). Thus, a sample size larger than 10

individuals is not be required to detect the signature of categorical perception.

Results

Bengalese finches show no evidence of categorical perception of carotenoid coloration

We first asked whether Bengalese finches label the Beak Set of colors in a way that is consistent with a

boundary between colors 5 and 6, as was found in zebra finches. In Bengalese finches, pass frequency

increased with increasing chromatic distance from the endpoint colors 1 or 8. Thus, color discrimination

improves as color pairs become more distinct from one another, with the mean (± standard deviation)

increase in pass frequency between neighboring colors (for example, the increase in pass frequency

from 1|2 to 1|3, or 1|3 to 1|4) being 0.12 ± 0.09. Across the color range, pass frequency increased more

between some color pairs than others, but in order for labeling to indicate a potential category

boundary, the largest increase should occur between the same colors in each direction. However, we

observed that for comparisons that included color 1, the largest increase occurred between 1|3 and 1|4

(0.25), while for comparisons including color 8, the largest increase in pass frequency occurred between

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6|8 and 5|8 (0.25) (Figure 2A). Thus, the labeling data did not suggest the presence of a category

boundary along the continuum. Additionally, the increase in pass frequency from 1|5 to 1|6 (0.12) was

smaller than that from 1|7 and 1|8 (0.22) and on par with the increase between 1|6 and 1|7 (0.10),

further indicating that the 5-6 color step, the location of a category boundary in zebra finches (Figure

2B), does not have special significance for the Bengalese finches.

The lack of evidence for a category boundary in the labeling data suggests that Bengalese

finches do not exhibit categorical perception within the Beak Set color range. Despite this, during

discrimination trials (those which use equally-spaced color pairs from across the continuum, rather than

comparisons with endpoint colors), Bengalese finches exhibited higher pass frequencies for some

equally-spaced color combinations than others. In particular, Bengalese finch ability to discriminate

between two colors generally increased as the Michelson contrast between two colors increased (Figure

3). In support of this, in Bengalese finches, the 'contrast' model, in which the predictor variable was

Michelson contrast of a given color comparison, was a substantially better fit than the 'category' model.

This result held whether considering color combinations that were 1, 2, or 3 color steps apart, as well as

for all comparisons combined (Table 1). In zebra finches, however, the 'category' model, in which the

predictor variable was a binary indicator of whether a comparison crossed the 5-6 boundary, was a

better fit in all cases, in line with our previous findings of categorical perception in this species.

In summary, for Bengalese finches, (1) labeling did not indicate the presence of a category

boundary; (2) the pattern of increased discrimination for some color pairs over others appears largely

attributable to differences in contrast; and (3) models suggested that contrast described the data better

than did a category boundary, unlike in zebra finches.

Contribution of chromatic distance and Michelson contrast to color discrimination

To further examine differences in how Bengalese and zebra finches discriminate colors, we used pass frequency data from the Extended Set of colors to build models in which pass frequency was predicted by chromatic distance and Michelson contrast in each species. We then applied the model fit to observed pass frequency in the Beak Set in two ways. We first visually examined their relationship to observed pass frequencies (Figure 4). Second, we used a modeling approach to examine how predicted pass frequency for a given comparison, whether or not that comparison included the 5-6 category boundary, and the interaction between those terms predicted observed color discrimination ability in each species (Table 2).

In Bengalese finches, observed pass frequency for all of the Beak Set color discrimination tasks was strongly correlated with expected pass frequency from the predictive model (i.e., the combination of chromatic distance and Michelson contrast), regardless of whether comparisons crossed the 5-6 boundary reported in zebra finches (Figure 4A, black line and points) or not (Figure 4A, gray solid line and points). There were nearly identical slopes of the lines for comparisons that either crossed (slope = 0.86) or did not cross (slope = 0.85) the 5-6 boundary, showing that the relationship between observed pass frequency and the pass frequency predicted by chromatic distance and contrast was the same for within- and between-category comparisons. Furthermore, a model showed that expected pass frequency explained a similar amount of variance regardless of whether the comparison crossed the boundary or not (crosses: R^2 = 0.79; does not cross: R^2 = 0.64). Therefore, though expected pass frequency strongly predicted observed pass frequency, the interaction between expected pass frequency and whether a comparison crosses the 5-6 boundary was not significant (Table 2).

By comparison, in zebra finches, we found clear differences between within- versus cross-boundary comparisons in terms of whether pass frequency was predicted by chromatic distance and Michelson contrast (Figure 4B). The slope of the line for comparisons that crossed (slope = 0.99) was

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different than the slope of the line for comparisons that did not cross the boundary (slope = 0.64).

Models showed pass frequency on cross-boundary comparisons (Figure 4B, black line and points) was

predicted very well by chromatic distance and Michelson contrast (R^2 = 0.92), whereas pass frequency

for within-category comparisons (Figure 4B, gray line and points) was not explained by the same

relationship between chromatic distance and Michelson contrast (R^2 = 0.19). Additionally, the

interaction term between expected pass frequency and crossing the 5-6 boundary was significant (p =

0.04; Table 2). This indicates a significant effect of crossing the boundary on how well color

discrimination ability can be predicted by Michelson contrast and chromatic distance.

Overall, we found clear differences between Bengalese finches and zebra finches in whether

their ability to discriminate between color pairs that do or do not cross the 5-6 boundary was predicted

by chromatic distance and Michelson contrast. In particular, Bengalese finch color discrimination was

predicted well by chromatic distance and Michelson contrast for both cross-boundary and within-

category comparisons. In zebra finches, however, whether or not the combination of chromatic distance

and Michelson contrast predicted pass frequency depended on whether that comparison involved

colors from opposite sides of the 5-6 boundary.

Discussion

Our results show differences in orange-red color perception in two related Estrildid finches that likely

have very similar spectral sensitivity (given the highly conserved spectral sensitivities of the

photoreceptors within the family Estrildidae; Figure S2, Hart et al. 2000), but which differ in their use of

color in signaling. In female Bengalese finches, tests of color discrimination ability showed no evidence

of categorical perception of orange-red coloration. Labeling tests did not indicate a category boundary

(Figure 2), and variation in discrimination of equally-spaced color pairs was explained better by the

Michelson contrast between colors than the presence of a category boundary (Figure 3, Table 1). This

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finding stands in contrast to female zebra finches, in which categorical perception of this same color range has been previously demonstrated (Caves et al. 2018), and in which discrimination of equally-spaced color pairs was better explained by a category boundary than by differences in Michelson contrast (Table 1). This finding is consistent with the hypothesis that perceptual processes may be adapted for signal function. In this case, zebra finches categorically perceive an orange-red range of colors that serve an important signal function in mate choice (Burley and Coopersmith 1987; de Kogel and Prijs 1996; Blount et al. 2003), whereas Bengalese finches, which do not exhibit carotenoid-based coloration and thus are unlikely to use orange-red coloration in a signaling context, do not exhibit categorical perception of this color range. Given that this is only a two-species comparison, a broader phylogenetic comparison (see, e.g., Figure S2) will be necessary to better understand the evolutionary implications of these results, specifically the extent to which the expression of categorical perception in Estrildid finches is linked to the use of color signals. A concordance between the use of color signals and the expression of categorical perception would support the hypothesis that the expression of categorical perception evolves as an adaptive response to the costs of assessing continuously varying traits (Green et al. 2020).

Comparing color discrimination between zebra finches and Bengalese finches yields some insight into the potential mechanisms underlying categorical color perception. In Bengalese finches, pass frequency on all color discrimination tasks was predicted by both chromatic distance and Michelson contrast (Figure 4A). In zebra finches, how well pass frequency was predicted by chromatic distance and Michelson contrast differed between within-category versus cross-boundary comparisons (Figure 4B). Thus, in zebra finches, it appears that the same sensory inputs—i.e. the same differences in hue and brightness— lead to different behavioral outputs depending on where two colors lie relative to the category boundary. For zebra finches, in nine out of 13 within-category comparisons, observed pass frequency was lower than predicted (Figure 4B) suggesting that categorical perception suppresses

within-category discrimination and enhances cross-boundary discrimination. By comparison, observed

pass frequencies were higher than predicted for 11 out of 12 between-category comparisons. This

finding is interesting in light of a recent study examining how the carotenoid concentration of oil

droplets found in zebra finch photoreceptors relates to categorical perception. In particular, individuals

with a higher concentration of carotenoids in their retinal oil droplets exhibited higher pass frequency

for cross-boundary color discriminations, but not within-category discriminations (Caves et al. 2020).

Together these results suggest that categorical perception may suppress color discrimination within

categories, and that aspects of retinal physiology such as oil droplet carotenoids, may serve as a

mechanism by which to enhance cross-boundary discrimination.

Filtering by carotenoid-containing oil droplets, however, has only a small impact on categorical

perception: Caves et al. (2020) found that less than 20% of variation in cross-boundary color

discrimination ability between individuals was attributable to variation in oil droplet carotenoids. More

broadly, data increasingly show that predictions of color discrimination based on the spectral sensitivity

of the photoreceptors alone (of which filtering by oil droplet carotenoids is a part) do not always align

with behavioral color discrimination data (e.g. Caves et al. 2018; Cheney et al. 2019; Zipple et al. 2019).

This is not surprising, given that many processes that occur after a stimulus is transduced by

photoreceptors affect perception. Additionally, behavioral response to color signals can be plastic

depending upon the developmental environment, as has been shown in juvenile zebra finches raised by

parents with manipulated bill coloration (ten Cate et al. 2006), though it is unclear if either perceptual

processing or sexual preferences were altered in this case. Given that color preferences can be plastic,

however, one line of future enquiry could investigate whether Bengalese finches possess the capacity to

develop categorical perception if they are exposed to the relevant carotenoid-based colors in their

developmental environment.

Our results may also contribute to our understanding of the mechanisms of color discrimination in birds, and in particular lend new insight into a recent analysis performed by Price et al. (2019). In particular, Price and colleagues focused on zebra finch color discrimination data reported in Caves et al. (2018) for comparisons from the Beak Set that are two color steps apart (color comparisons 1|3, 2|4, 3|5, 4|6, 5|7, and 6|8). They noted a close fit between observed zebra finch discrimination data and the predicted discriminability of color pairs if zebra finch color discrimination is based on luminance contrast, i.e. the photon catch of the double cones. However, the zebra finch discrimination data deviate from the predictions based on luminance contrast close to the category boundary (Figure 5, gray versus dashed lines). First, in the behavioral data from Caves et al. (2018), pass frequency increases less from 4|6 to 5|7 than the luminance model of Price et al. (2019) predicts, because the luminance model predicts that birds should pass at much higher frequency for 5|7 (Michelson contrast ~0.4) than for 4|6 (Michelson contrast ~0.2). Second, behavioral pass frequency decreases more from 5|7 to 6|8 than predicted by the luminance model, with behavioral pass frequency for 6|8 (a relatively high-contrast comparison) on par with pass frequency for 1|3, 2|4 and 3|5, where Michelson contrast is approximately zero. These deviations from the expectations from Price et al. (2019) are consistent with our model results (Table 1), which indicate that zebra finch color discrimination is better predicted by a category boundary than by luminance differences. Behavioral data from the Bengalese finches, however, in which brightness differences play a critical role in discrimination, line up very closely with luminance contrast-based predictions of discriminability from Price et al. (2019) model (Figure 5, black line versus dashed line). Thus, one possibility is that luminance contrast underlies color discrimination in birds, but that processes like categorical perception can further modify how color is perceived.

In conclusion, the Bengalese finch, a species lacking carotenoid-based coloration, does not discriminate colors along an orange-red continuum in a categorical fashion. This is in contrast to the zebra finch, for which this color range plays an important role in signaling and which expresses

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categorical perception of colors along the same continuum. This finding is consistent with the idea that selection on a signaling system may act on the perceptual mechanisms underlying that system. However, a more comprehensive phylogenetic analysis of the relationship between signal expression and differences in perception is needed to better understand how perceptual processes evolve in the context of signaling, and the ways in which signal receivers and senders may place selective pressures on one another.

Statement of Authorship

EMC, PAG, SP, SJ, and SN conceived of the study. SN acquired funding. EMC, PAG, and SJ developed the methods and designed the experiment. EMC, PAG, and DB collected the data. MNZ led the data analysis, model analysis, coding, and visualization with EMC. SP and SN provided resources. EMC wrote the original draft. All authors reviewed and edited the manuscript.

Data and Code Accessibility

Data and codes supporting the results are archived in the Dryad Data Repository (https://doi.org/10.5061/dryad.stqjq2c1z; Caves et al. 2021).

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Tables

Table 1. Model fits for linear mixed models that predict pass frequency on 1-, 2-, or 3-apart discrimination tasks using either the Michelson contrast or a binary indicator of whether or not that comparison crosses the 5-6 boundary found in zebra finches and include bird ID as a random effect. The combined models contain data from all discrimination trials (1-, 2-, and 3-aparts) and additionally include chromatic distance as a fixed effect predictor. For each model pair, the better-fit model is listed first. AIC is the Akaike Information Criterion, and ΔAIC is calculated relative to the best-fit model. l_i is the relative likelihood of model i, and w_i is the probability that it is the best model within a set.

		R^2	AIC	ΔAIC	l_i	w_i	Interpretation
Zebra Finches							
1 apart	Category	0.4	-15.7	0	1.0	0.60	Categorical Model Better
	Contrast	0.33	-14.9	0.8	0.67	0.40	
2 apart	Category	0.90	-15.3	0	1.0	0.96	Categorical Model Better
	Contrast	0.70	-8.7	6.6	0.04	0.04	
3 apart	Category	0.99	-24.6	0	1.0	0.99	Categorical Model Better
	Contrast	0.92	-13.3	11.3	<0.01	<0.01	
Combined	Category	0.84	-46.2	0	1	0.91	Categorical Model Better
Model	Contrast	0.80	-41.6	4.6	0.1	0.09	
Bengalese Finches							
1 apart	Contrast	0.41	-17.5	0	1.0	0.86	Contrast Model Better
	Category	0.005	-13.9	3.6	0.17	0.14	
2 apart	Contrast	0.95	-20.8	0	1.0	0.99	Contrast Model Better
	Category	0.79	-11.6	9.2	0.01	0.01	
3 apart	Contrast	0.88	-10.5	0	1.0	0.85	Contrast Model Better
	Category	0.76	-7.0	3.5	0.17	0.15	
Combined	Contrast	0.87	-43.2	0	1.0	0.99	Contrast Model Better
Model	Category	0.74	-31.2	12.0	<0.01	<0.01	

Table 2. The relationship between expected and observed pass frequencies for comparisons that do or do not include the 5-6 boundary, in Bengalese and zebra finches. The "5-6 boundary" term is a binary indicator of whether or not a given comparison included the 5-6 boundary.

Species	Parameter	Coefficient	Std. Error	p value	Interpretation
Bengalese Finches	Intercept	-0.026			Observed pass frequency is predicted by expected pass frequency (i.e. chromatic distance + Michelson contrast) for all comparisons
	Expected Pass Frequency	0.85	0.22	0.0008	
	5-6 Boundary	-0.02	0.10	0.84	
	Interaction Between Expected Pass Frequency and 5-6 Boundary	0.007	0.25	0.98	
Zebra Finches	Intercept	0.09			Observed pass frequency is predicted by expected pass frequency (i.e. chromatic distance + Michelson contrast) only for those comparisons that cross the 5-6 boundary
	Expected Pass Frequency	0.44	0.23	0.08	
	5-6 Boundary	-0.04	0.07	0.62	
	Interaction Between Expected Pass Frequency and 5-6 Boundary	0.56	0.26	0.04	

Figure Legends

Figure 1. The locations of selected colors in avian chromaticity space. Circles and solid lines indicate the Beak Set, while squares and dashed lines indicate the Extended Set. The gray shading of an icon indicates its Munsell value. Colors range from 1 (the most red) to 8 (the most orange), and numbers marked with a * are the four new colors that were used in the Extended Set.

Figure 2. Labeling data for the Beak Set in (A) Bengalese finches and (B) zebra finches. (A) Female Bengalese finches showed no evidence of labeling consistent with categorical perception, in that the largest increases in pass frequency (arrows) occurred in different parts of the color continuum in each direction. (B) Female zebra finches show the characteristic labeling curve for categorical perception, with the largest increases in pass frequency (arrows) being in the same place, between colors 5 and 6, in each direction (data from Caves et al. 2018). The solid line represents pass frequency for 1|# trials; the dashed line represent results for #|8 trials. Vertical bars with each dot represent standard error. The dashed gray line indicates expected pass frequency if birds flip discs at random. A color version of this figure is available online.

Figure 3. Bengalese finch discrimination of Beak Set color stimuli that are one, two, or three color steps apart. Within each comparison type, boxes are ordered from left to right by increasing Michelson contrast (shown in brackets). For each comparison, the median (horizontal line), 25th and 75th percentiles (boxes), and 1.5x interquartile range (whiskers), for individual pass frequency are presented.

Figure 4. Observed pass frequency versus expected pass frequency for comparisons that do (black) and do not (gray) cross the 5-6 boundary in (A) Bengalese and (B) zebra finches. Each dot is a color

comparison from the original Beak Set. The dashed line has a slope of 1, i.e. represents the expected

relationship if observed and expected pass frequency are identical.

Figure 5. Behavioral color discrimination data for two-apart comparisons for zebra finches (solid gray

line) and Bengalese finches (solid black line), as well as predicted pass frequency from a model of color

discrimination based on luminance contrast information (dashed line). Data for zebra finches are

originally from Caves et al. (2018), and data from Bengalese finches are presented here. The model

predictions are redrawn from Price et al. (2019), figure 4b. Price et al. state that the y-axis values for

their predictions are unknown, because they depend on how the inputs from each photoreceptor type

are weighted, but that the shape of the predicted curve is of more importance than its height. Thus,

their prediction curve is scaled here so that the maximum pass frequency aligns with the observed

maximum mean pass frequency in our studies.

Figure 1

Figure 2

Figure 3

Figure 4

Figure 5

Supplementary Files (PDF, Word, TeX, figures)

Supplemental tables and figures for: Eleanor M. Caves[1,2], Patrick A. Green[1,2], Matthew N. Zipple[2], Dhanya Bharath[3], Susan Peters[2], Sönke Johnsen[2], and Stephen Nowicki[2,4]. Comparison of categorical color perception in two Estrildid finches. The American Naturalist.

[1]Centre for Ecology and Conservation, College of Life and Environmental Sciences, University of Exeter, Penryn, Cornwall TR10 9FE, UK; [2]Biology Department, Duke University, Durham, NC 27708, USA; [3]Indian Institute of Science, Bengaluru, Karnataka 560012, India; [4]Department of Neurobiology, Duke University Medical School, Durham, NC 27708, USA

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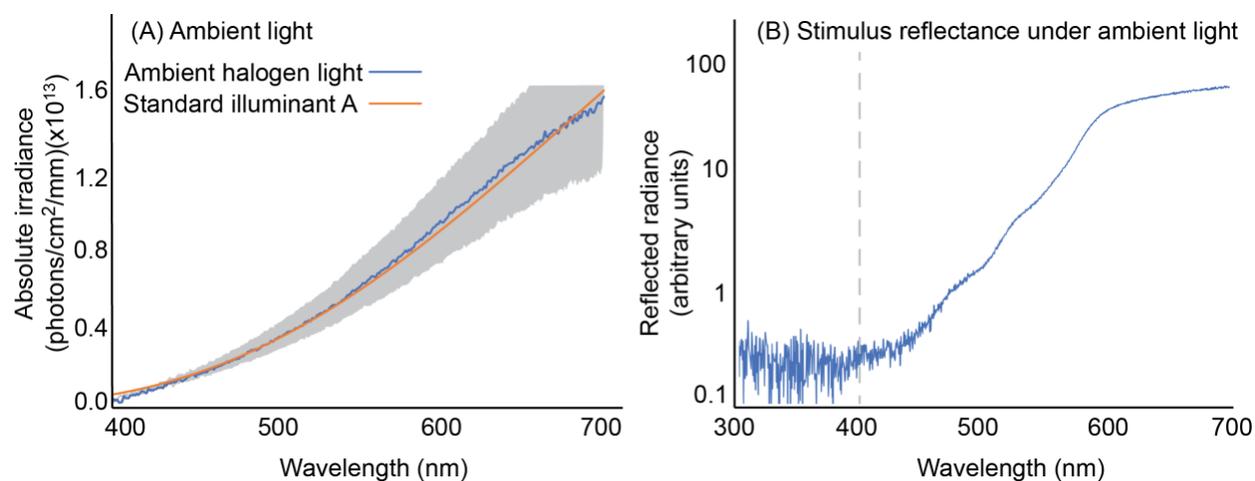

Figure S1. Lighting conditions in the experimental rooms. (A) Mean absolute irradiance of the ambient lighting conditions in the experimental rooms (blue line) almost exactly match Standard Illuminant A (orange line), which we used in our calculations of chromatic distance in order to maximize the ability of other researchers to reproduce our work. The gray area represents one standard deviation in either direction, as these measurements were taken from 20 different bulbs. (B) Under ambient light, reflectance from our chip stimuli (Munsell paper covered with an epoxy cover) in the UV range (below 400nm; to the left of the gray dashed line) was so low as to reach to the noise floor of the spectrometer, suggesting that our use of a trichromatic visual system to calculate chromatic distance is appropriate in this case. Figures adapted from Caves et al. (2018).
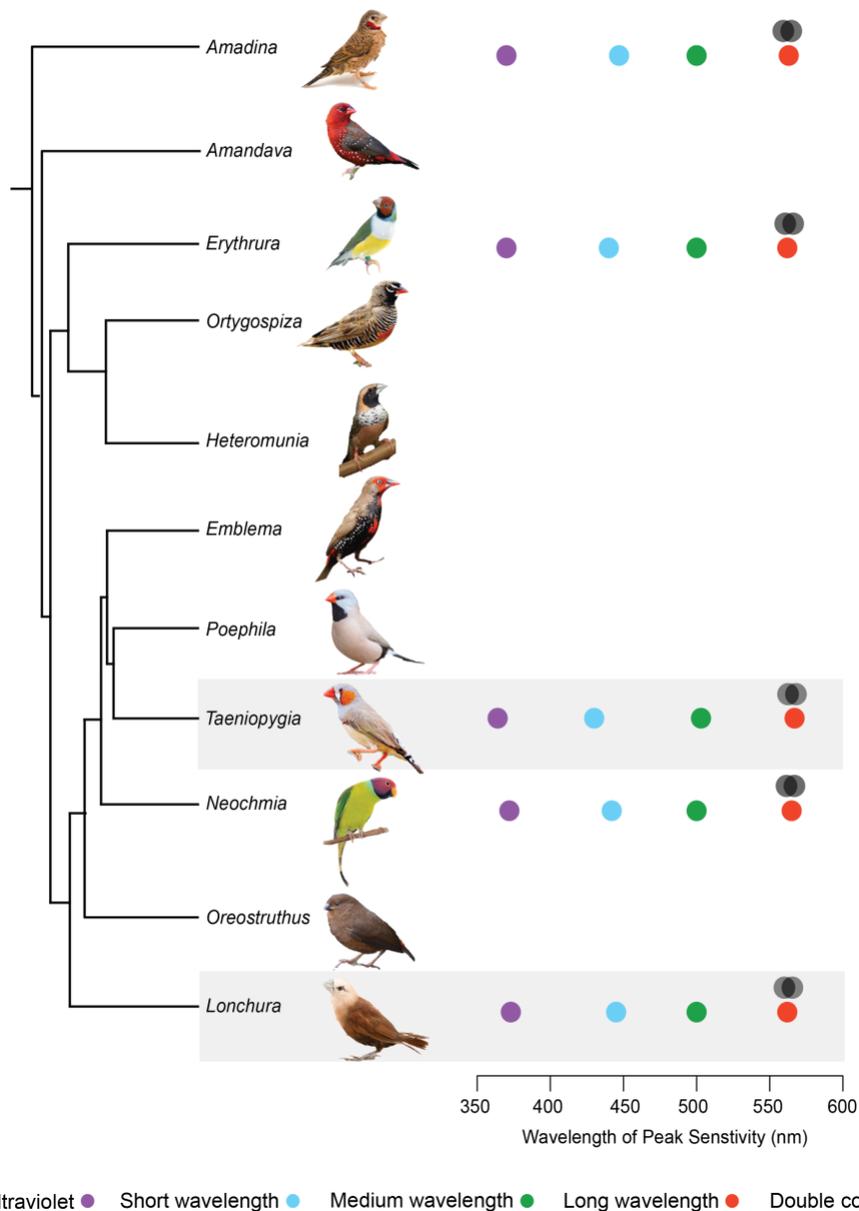
Figure S2. Relationships among select genera in the family Estrildidae (phylogeny based on Olsson and Alström 2020) and spectral sensitivity data for those species whose photoreceptor sensitivities have been measured (data from Bowmaker et al. 1997; Hart et al. 2000; Lind 2016). Spectral sensitivity is given as wavelength of peak sensitivity in the double cones (black double circles) and the four single cones (circles): ultraviolet (purple), short wavelength sensitive (blue), medium wavelength sensitive (green), and long wavelength sensitive (red). The double cone and long wavelength-sensitive single cone are shown vertically offset from one another due to overlap in peak sensitivity. For each genus, a representative member is pictured, to illustrate patterns of coloration among genera (images from Wikimedia commons). For genera in which a species has known spectral sensitivity (*Amadina fasciata, Erythrura gouldiae, Neochmia modesta, Taeniopygia guttata,* and *Lonchura maja*), the measured species is pictured. Gray shaded areas denote the two genera examined in this study, *Taeniopygia* and *Lonchura*.

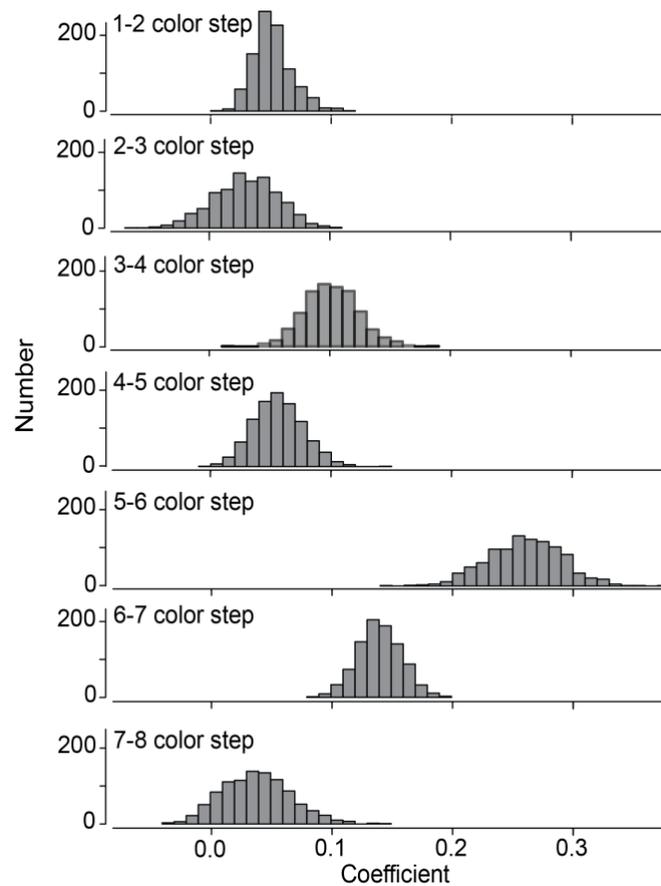**Figure S3. Results of randomly sampling data from 26 female zebra finches (data from Caves et al. 2018) to a sample size of n=10, to determine whether lack of evidence for categorical perception in Bengalese finches was due simply to having a lower sample size.** The x-axis coefficients were generated by a linear mixed effects model (built in *lme4*, Bates et al. 2015) that combined data from zebra finch labelling and discrimination trials (described in detail in Zipple et al. 2019). The model included pass frequency for a given comparison as the response variable, each of the seven color steps as binary fixed effects, and bird ID as a random effect. In brief, this model estimated the effect of each color step on pass frequency, independent of other color steps. For example, a two-apart trial with 1|3 bicolor discs includes steps 1-2 and 2-3, while a three-apart trial using 1|4 bicolor discs includes steps 1-2, 2-3, and 3-4. The difference in a bird's pass frequency in 1|4 trials as compared to 1|3 trials therefore provides information that helps the model estimate the effect of specifically crossing the 3|4 boundary.

To create the data shown above, we randomly sampled ten individual zebra finches from our dataset of 26 individuals, and calculated the model coefficients for each color step. We then repeated the random sampling 1000 times, each time calculating the model coefficients, to generate a distribution of the effects of each color step for different combinations of randomly selected individuals. As can be seen, in nearly all cases, the effect of the 5-6 color step was still much larger than any other, meaning that no matter which ten zebra finches are selected, there is still evidence for categorical perception.

Table S1. Relative photon (quantum) catch values for UV-, short-, medium-, and long-wavelength sensitive cones, as well as the double cone, of the zebra finch. Values were calculated using the reflectance spectrum of each Munsell color, spectral sensitivities of the zebra finch single and double cones, and standard illuminant A. For Munsell colors, the hue group and saturation/chroma are given.

Color	Munsell color	UV	S	M	L	Double
1	5R 4/4	1.22E+12	2.36E+13	6.43E+13	1.99E+14	9.70E+13
2	5R 4/6	1.15E+12	2.06E+13	6.05E+13	2.30E+14	1.05E+14
3	7.5R 4/6	9.77E+11	1.91E+13	5.94E+13	2.33E+14	1.04E+14
4	7.5R 4/8	1.00E+12	1.80E+13	5.77E+13	2.78E+14	1.13E+14
5	10R 4/8	7.67E+11	1.48E+13	5.71E+13	2.79E+14	1.14E+14
6	10R 5/10	9.76E+11	1.82E+13	8.37E+13	4.37E+14	1.75E+14
7	10R 6/12	1.33E+12	2.37E+13	1.24E+14	6.90E+14	2.67E+14
8	2.5YR 6/12	1.09E+12	2.01E+13	1.29E+14	6.37E+14	2.57E+14
2*	5R 5/6	1.72E+12	3.28E+13	9.31E+13	3.36E+14	1.54E+14
3*	5R 6/8	2.29E+12	4.35E+13	1.31E+14	5.59E+14	2.39E+14
4*	7.5R 5/8	1.30E+12	2.52E+13	8.55E+13	3.77E+14	1.60E+14
8*	10R 5/12	8.35E+11	1.51E+13	7.88E+13	4.86E+14	1.84E+14

Table S2. Chromatic distances between colors in the Beak color set were robust to differences in visual system and ambient light parameters used. Here we show chromatic distances between color pairs calculated using (1) zebra finch *Taeniopygia guttata* UVS cone-type retina and standard Illuminant A; (2) zebra finch spectral sensitivity, ambient (halogen) light spectrum from experimental trials; (3) starling *Sturnus vulgaris* UVS cone-type retina and standard Illuminant A; (4) the average avian UVS cone-type retina with standard Illuminant A; (5) the average avian VS cone-type retina with standard Illuminant A; and (6) zebra finch UVS cone-type retina and standard illuminant A in a tetrachromatic scenario, i.e. incorporating the UV cone catch.

Condition	1v2	2v3	3v4	4v5	5v6	6v7	7v8
(1) Zebra Finch, Illuminant A	4.2	2.7	4.3	4.7	4.2	2.8	4.7
(2) Zebra finch, halogen experimental lighting	4.3	2.7	4.3	4.7	4.2	2.8	4.6
(3) Starling, Illuminant A	3.8	1.8	3.5	3.5	3.5	2.1	2.7
(4) Average UVS, Illuminant A	3.8	1.8	3.5	3.5	3.5	2.1	2.7
(5) Average VS, Illuminant A	4.3	2.1	3.5	3.1	3.7	2.3	2.1
(6) Zebra Finch, Illuminant A, Tetrachromatic	4.2	2.7	3.6	4.7	3.9	2.9	3.7

Table S3. Comparisons included in the predictive model, i.e. 13 comparisons that do not include the 5-6 boundary. As in Figure 1, numbers marked with a * are the four new colors that were used in the Extended set.

Comparison	Contrast	Chromatic Distance
1v2*	0.23	2.9
1v3*	0.42	6.2
1v4*	0.25	8.2
1v5	0.08	11.3
2*v3*	0.22	3.3
2*v4*	0.02	5.4
2*v5	0.15	8.6
3*v4*	0.20	2.3
3*v5	0.35	5.4
4*v5	0.17	3.2
6v7	0.21	2.8
6v8*	0.03	4.2
7v8*	0.18	1.7

Table S4. Results of the fixed-intercept linear models of pass frequency generated from birds' performance on comparison of colors in the Extended color set. Coefficient estimates from these models were used to generate expected pass frequencies for the Beak dataset.

Species	Parameter	Coefficient Estimate	Standard Error	t value	p value	R^2 of model
Bengalese Finch	Michelson Contrast	0.97	0.14	7.2	**<0.0001**	**0.96**
	Chromatic Distance	0.02	0.01	4.4	**0.001**	
Zebra Finch	Michelson Contrast	0.53	0.13	4.2	**0.001**	**0.91**
	Chromatic Distance	0.02	0.01	3.4	**0.006**	

Table S5. Performance of models of pass frequency for color comparisons from the Extended color set. Note that in both species, the model that included both chromatic distance and Michelson contrast performed much better than models that included only one or the other.

Species	Model	AIC	ΔAIC to Best Model
Bengalese Finch	Chromatic Distance + Michelson Contrast	-29.1	0
	Contrast Only	-21.7	7.4
	Chromatic Distance Only	-18.6	11.5
Zebra Finch	Chromatic Distance + Michelson Contrast	-27.5	0
	Contrast Only	-16.4	11.1
	Chromatic Distance Only	-6.8	20.7

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Lind, O. 2016. Colour vision and background adaptation in a passerine bird, the zebra finch (*Taeniopygia guttata*). Royal Society Open Science 3:160383.

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