Wild hummingbirds discriminate nonspectral colors

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Many animals have the potential to discriminate nonspectral colors. For humans, purple is the clearest example of a nonspectral color. It is perceived when two color cone types in the retina (blue and red) with nonadjacent spectral sensitivity curves are predominantly stimulated. Purple is considered nonspectral because no monochromatic light (such as from a rainbow) can evoke this simultaneous stimulation. Except in primates and bees, few behavioral experiments have directly examined nonspectral color discrimination, and little is known about nonspectral color perception in animals with more than three types of color photoreceptors. Birds have four color cone types (compared to three in humans) and might perceive additional nonspectral colors such as UV+red and UV+green. Can birds discriminate nonspectral colors, and are these colors behaviorally and ecologically relevant? Here, using comprehensive behavioral experiments, we show that wild hummingbirds can discriminate a variety of nonspectral colors. We also show that hummingbirds, relative to humans, likely perceive a greater proportion of natural colors as nonspectral. Our analysis of plumage and plant spectra reveals many colors that would be perceived as nonspectral by birds but not by humans: Birds' extra cone type allows them not just to see UV light but also to discriminate additional nonspectral colors. Our results support the idea that birds can distinguish colors throughout tetrachromatic color space and indicate that nonspectral color perception is vital for signaling and foraging. Since tetrachromacy appears to have evolved early in vertebrates, this capacity for rich nonspectral color perception is likely widespread.

color vision | tetrachromacy | signal evolution | hummingbirds | sensory ecology

For many animals, color vision is essential for finding food, selecting mates, avoiding predators, provisioning young, and navigating through varied landscapes. How does color vision work? Across the animal kingdom, species vary widely in the number and spectral sensitivities of color photoreceptor types in their eyes (1). However, it is not the number of photoreceptor types but how they interact that determines the "dimensionality" of an animal's color vision. In vertebrates, the photoreceptors responsible for color vision are the cones. If an animal possesses two interacting color cone types (i.e., there is a neural comparison of their outputs), it is a dichromat, with two dimensions of vision. It can discriminate lightness (intensity) and one dimension of hue/saturation. Two appropriately chosen monochromatic lights (or primaries) are necessary and sufficient to match any color a dichromat can see. Typically, a trichromat has three interacting cone types, tetrachromats have four, and so on (2), with color vision stemming from neural comparisons of all three, four, or more cone types, respectively.

Understanding this dimensionality is key to validating color vision models that predict how animals see the world (2–5). Humans have trichromatic color vision: All of the colors we can see result from neural comparisons of all three retinal color cone types, and three primaries are necessary and sufficient to match any color. Birds, by contrast, possess a fourth color cone type, which is sensitive to UV wavelengths (6, 7). They are thought to have tetrachromatic color vision, involving interactions of their UV- or violet-sensitive (UVS/VS), short-wave–sensitive (SWS), medium-wave–sensitive (MWS), and long-wave–sensitive (LWS) color cones. Indirect evidence for avian tetrachromacy comes from the general agreement of behavioral data with a model that predicts discrimination thresholds from opponent signals stemming from four single color cone types (8, 9). More directly, color-matching experiments (10) and tests designed to stimulate specific photoreceptors (11, 12) have suggested that avian color vision results from at least three different opponent mechanisms (reviewed in ref. 2). This means that signals originating in the four color cone types are likely combined (they interact) in at least three opponent processes that compare the outputs of spectrally adjacent cone types (10): UVS vs. SWS, SWS vs. MWS (or SWS vs. MWS+LWS; see ref. 11), and MWS vs. LWS. Two cone types are said to be spectrally adjacent if they are sensitive to wavelengths in nearby ranges of the light spectrum (e.g., the UVS and SWS cones are sensitive to UV and short wavelengths, respectively, which fall in neighboring portions of the light spectrum).

Significance

Birds have four color cone types in their eyes, compared to three in humans. In theory, this enables birds to discriminate a broad range of colors, including many nonspectral colors. Nonspectral colors are perceived when nonadjacent cone types (sensitive to widely separated parts of the light spectrum) are predominantly stimulated. For humans, purple (stimulation of blue- and red-sensitive cones) is a nonspectral color; birds' fourth color cone type creates many more possibilities. We trained wild hummingbirds to participate in color vision tests, which revealed that they can discriminate a variety of nonspectral colors, including UV+red, UV+green, purple, and UV+yellow. Additionally, based on an analysis of ~3,300 plumage and plant colors, we estimate that birds perceive many natural colors as nonspectral.

EVOLUTION

In theory, the increased dimensionality of color vision (relative to humans) contributes to an expansion of hues perceptible to birds (13, 14), the full range of which can be represented in a color space, a classic model of color vision. The avian tetrahedral color space is a type of chromaticity diagram (5), in which any bird-visible color can be mapped as a point defined by the relative stimulation of the four color cone types (Fig. 1A). A fundamental assumption of tetrachromacy is that birds can distinguish colors throughout this entire space: colors resulting from interactions of all four color cone types, not just by interactions of spectrally adjacent cone types (discussed above) but also by interactions of nonadjacent cone types (Fig. 1A), which by definition (see below) generate nonspectral color perception (3, 13).

Monochromatic colors in the rainbow are often called spectral colors. In terms of perception, spectral colors arise when light predominantly stimulates a single color cone type or two spectrally adjacent cone types. For humans, red and yellow are examples of spectral colors because light predominantly stimulates the LWS (red) cone type (for red) or the spectrally adjacent MWS (green) and LWS (red) cone types (for yellow). In avian color space, spectral colors are represented on three axes comprising the spectral/monochromatic line (the colored line in Fig. 1A). Nonspectral colors are not part of the rainbow (i.e., cannot be evoked by monochromatic light). Technically speaking, any color not on the spectral line might be considered "nonspectral." Here, however, we follow conventions in the literature (3, 4, 13, 15, 16) and use a narrower definition of "nonspectral color" to denote a type of perceived color that results from the predominant stimulation of nonadjacent cone types (Fig. 1 A and C). We elaborate on this definition in *Materials and Methods*. Using this definition, humans perceive just one such nonspectral color: purple (17, 18), which arises when our SWS (blue) and LWS (red) cones are simultaneously
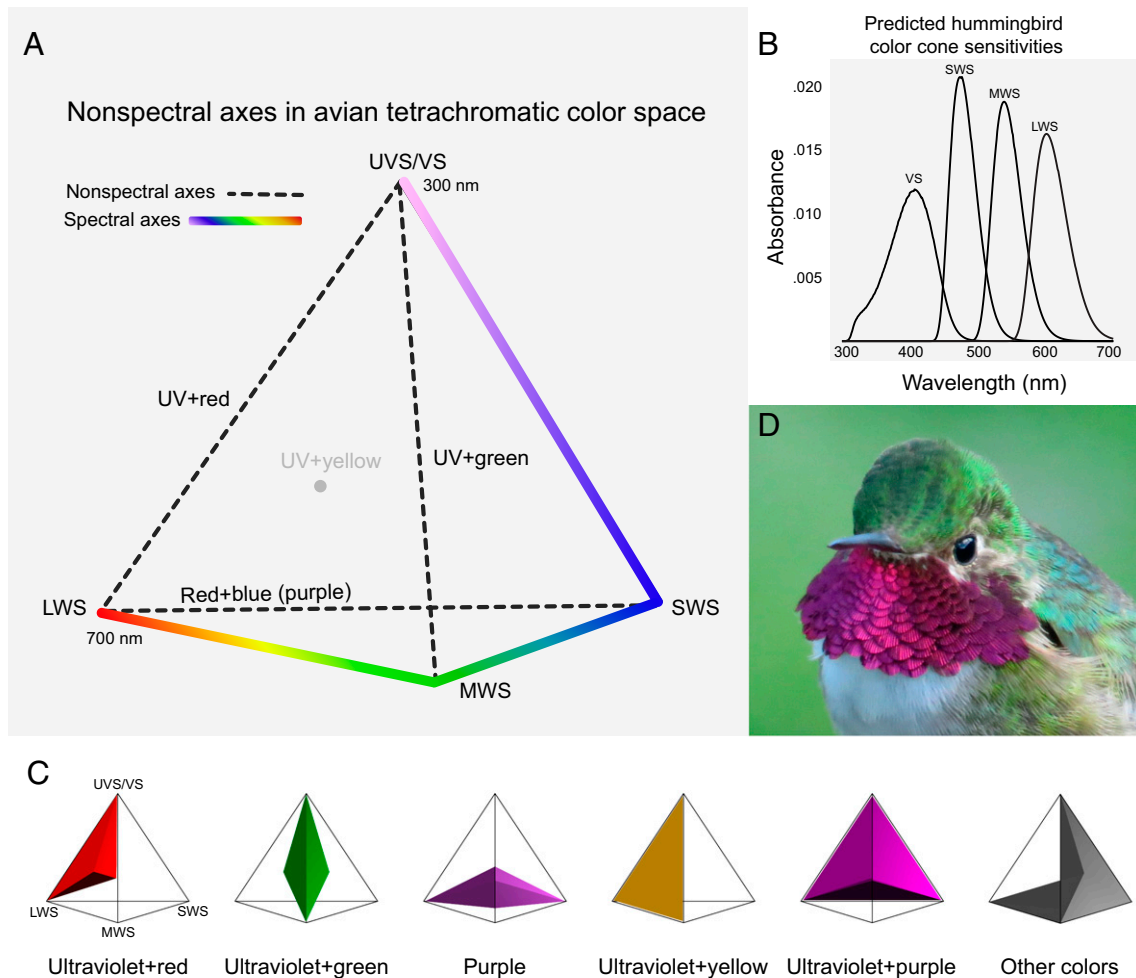
Fig. 1. Nonspectral color perception and the dimensionality of avian vision. (*A* and *B*) Birds typically possess four color cone types in their retinas, sensitive to long (long-wave–sensitive [LWS]), medium (MWS), short (SWS), and UV/violet (UVS/VS) wavelengths. Hummingbirds are believed to possess the VS-type visual system; their predicted color cone sensitivities are shown in *B*, from ref. 37. For simplicity, we refer to colors that would stimulate the UVS or VS cones as "ultraviolet." The avian color space (*A*), which theoretically captures the full range of perceivable colors, can be represented in a tetrahedron. This depiction, which does not include information about luminance, is called a chromaticity diagram. Each vertex represents one of the four color cone types. Previous work (see main text) has shown that birds can discriminate colors along the three spectral axes, shown in color in *A*, which represent the different colors in the bird-visible spectrum of light. However, little is known about the extent to which birds can discriminate colors along the three nonspectral axes—UV+red, UV+green, and red+blue (purple), illustrated with dashed lines in *A*. Whereas the nonspectral axes (dashed lines) represent secondary (two-cone) nonspectral colors, UV+yellow is a ternary (three-cone) nonspectral color, mostly stimulating the UVS/VS, MWS, and LWS cones. UV+purple, mostly stimulating the UVS/VS, SWS, and LWS cones, is another type of ternary nonspectral color. The regions of color space occupied by these nonspectral colors are illustrated in *C*. The region of color space shaded in gray represents colors that do not meet our definition of nonspectral. We predict that birds are able to perceive a variety of nonspectral colors. We investigated this in a population of wild broad-tailed hummingbirds (*D*). Shown here is a male. The magenta gorget feathers are likely perceived as nonspectral by birds (see Fig. 2 *A*, *f*).

stimulated, while the MWS (green) cone is not substantially stimulated. In avian color space, secondary (two-cone) nonspectral colors are represented on the three axes not forming the spectral line. An avian tetrachromat might have five types of nonspectral colors (15, 16). These include three secondary (two-cone) nonspectral colors: "ultraviolet+red" (UVS + LWS), "ultraviolet+green" (UVS + MWS), and "purple" (SWS + LWS); and two ternary (three-cone) nonspectral colors: "ultraviolet+purple" (UVS + SWS + LWS) and "ultraviolet+yellow" (UVS + MWS + LWS) (Fig. 1 A and C).

Can birds discriminate nonspectral colors like UV+red and UV+green? Outside of work on trichromatic primates (18, 19) and bees (20), few detailed behavioral experiments on nonspectral color perception have been conducted (14). A rare exception to this is a study demonstrating that budgerigars (*Melopsittacus undulatus*) can distinguish yellow light from a mixture of UV+yellow light (10). The extent to which birds and other potentially tetrachromatic animals can perceive and discriminate nonspectral colors thus remains an open question (3, 4, 13, 14). This is significant because many animals, including many fish, reptiles, and dinosaurs, are thought to have (or have had) the capacity for tetrachromatic vision. This kind of color vision system likely evolved in early diurnal vertebrates, which possessed four types of visual pigment opsins in their cone photoreceptors (21). Given the importance of color vision—and the evolution of colorful signals—in the context of foraging, mate choice, social signaling, and predator–prey interactions across vertebrate animals (22), it is surprising that we know so little about nonspectral color discrimination. To appreciate how tetrachromatic color vision works, deciphering the extent to which animals perceive nonspectral colors is critical.

Behavioral experiments on bird color vision have proliferated in recent decades. In the laboratory, researchers have employed the tools of psychophysics to determine color discrimination thresholds, typically using carefully calibrated artificial stimuli (e.g., narrow-band monochromatic lights or broader-band colored disks, paper cones, or computer monitors) (9, 23, 24). Although the colors examined in this context have been diverse, we are not aware of any study specifically investigating how birds discriminate colors that fall on or near the nonspectral axes of avian color space (Fig. 1A). A separate line of behavioral research, usually conducted in the field or in seminatural settings, has explored how birds evaluate the colors of natural objects such as fruit, flowers, prey items, plumage, and eggs, typically in the contexts of foraging, mate choice, and defense against predators and brood parasites. Some of these natural colors are likely perceived as nonspectral by birds; see, for example, the UV+red and UV+yellow wing colors of *Heliconius* butterflies in ref. 25. However, experiments of this kind were not designed to test nonspectral color perception. Consequently, to determine whether birds can behaviorally distinguish nonspectral colors throughout the tetrahedral color space (Fig. 1 A and C), systematic psychophysical experiments are required.

Despite the surge in animal color research (22, 26), a major challenge is that most "bottom-up" (24) work on the proximate mechanisms of color perception is performed under unnatural conditions. Psychophysical experiments are usually conducted on a few captive individuals in an indoor laboratory. While these experiments have been vital for developing color vision models (5, 24), they lack ecological context and may fail to capture behaviors that are biologically relevant. Experience with prey items (27), fruit (28), and flowers (29) can influence how birds learn colors, suggesting that the perceptual and cognitive experiences of wild birds may be different from those of birds reared and studied in the laboratory. In addition, color perception itself might depend on the light conditions birds experience during development, since the sensitivities of oil droplets (tiny organelles in the cones that can filter light) can vary with ambient light intensity (30). In short, laboratory experiments are essential, but they must be complemented with comprehensive field experiments if we wish to understand how wild animals use color vision in the real world—in complex habitats and under changing conditions. Without field-based psychophysical studies, we run the risk of misinterpreting the ecology and evolution of colorful signals, which are shaped by the perceptual experiences and behavioral responses of wild animals.

Hummingbirds (Aves: Trochilidae) are an excellent candidate group for studying color vision in the wild. Because they evolved to respond to colorful floral advertisements of nectar, hummingbirds can be easily trained to associate different colors with a reward (31–33) and have proven to be outstanding for examining cognition outside the laboratory (29, 31). However, except for early work on black-chinned hummingbirds (*Archilochus alexandri*) (34–36), they have not been used to investigate mechanisms of color perception in a natural setting.

In this study, we established a field system for investigating color perception in a wild population of broad-tailed hummingbirds (*Selasphorus platycercus*). We designed and deployed calibrated, field-portable light-emitting diode (LED) devices, the TETRACOLORTUBES, which can be programmed to display a broad range of bird-visible colors. To test the hypothesis that hummingbirds can discriminate a variety of nonspectral colors, we used an experimental paradigm in which birds were trained to associate a color with a rewarding sucrose solution. In addition, we used a large dataset of plumage and plant reflectance measurements to estimate the proportion of colors in the natural environment that may be perceived by birds as nonspectral.

Can Wild Hummingbirds Discriminate Nonspectral Colors?

We conducted a series of field experiments on nonspectral color discrimination in a population of wild, free-flying broad-tailed hummingbirds (Fig. 1D) near the Rocky Mountain Biological Laboratory (RMBL) (~2,920-m elevation) in Gothic, Colorado, during three field seasons (June 2016, 2017, and 2018). Extending the approach of Goldsmith and colleagues (34–36), we trained hummingbirds to associate one color (the reward color) with a sucrose solution and a second color (the nonreward color) with water (*Materials and Methods*). Each experiment tested discrimination between two colors and consisted of multiple trials, performed sequentially. For each trial, we recorded the number of primary visits (*Materials and Methods*) to each color. Colors were produced by two TETRACOLORTUBES (see below; *SI Appendix*, Fig. S1). The two tubes were placed on adjacent tripod platforms (~1 m apart and ~1.4 m above the ground) (Fig. 2C). Each platform supported a tube and a clear plastic saucer partially filled with either sucrose solution or water. When a hummingbird entered the experimental arena (defined as within ~4 m of the platforms), an observer ~10 m away recorded whether the bird first visited the reward or nonreward color. Because hummingbirds can readily learn location cues (34), after each trial we swapped the locations of the reward (sucrose saucer with reward color) and nonreward (water saucer with nonreward color) setups to prevent birds from cueing on the spatial location of the reward.

We performed experiments throughout the day (typically between 06:30 and 19:30) in a field near a small wooden building. Experiments ranged from 2 to 11.5 h, with approximately 100 to 700 recorded visits per experiment. Fluctuating conditions (weather, floral abundance) throughout the field season, plus interannual variation in population dynamics, contributed to variation in length and sample size across experiments. We individually marked a fraction of the population, enabling us to estimate the population size ($n = 281$ in 2017; $n = 198$ in 2018) in Gothic and its immediate surroundings (see *SI Appendix*: "Marking individual birds" and "Estimating population size"). These population numbers represent the inferred number of individuals visiting our field site, not the number of individuals visiting during each experiment. We do not know exactly how many individuals

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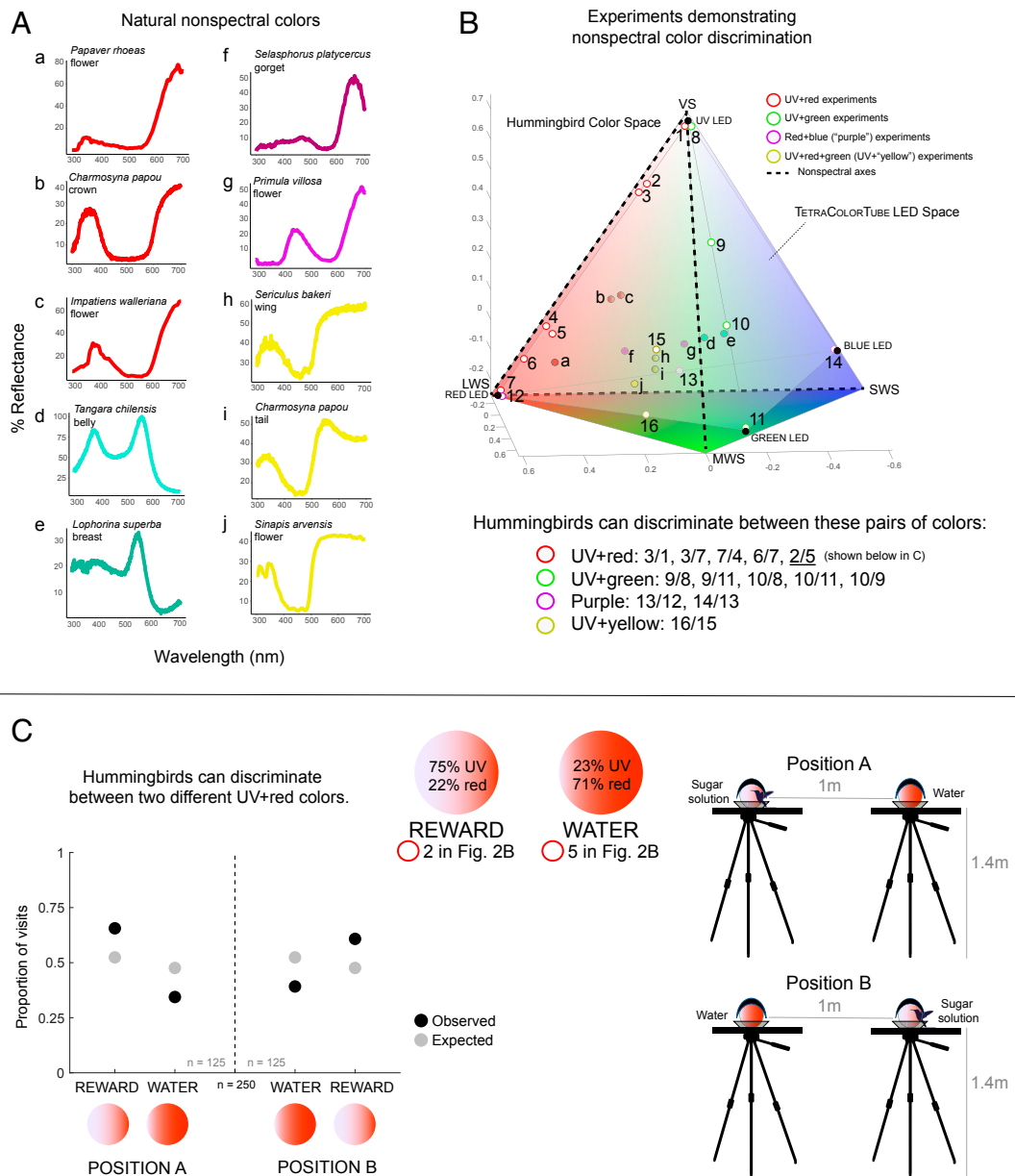
Fig. 2. Wild hummingbirds can discriminate nonspectral colors. (*A*) A range of natural plant and plumage colors that would likely appear ultraviolet+red (*a*, *b*, and *c*), ultraviolet+green (*d* and *e*), purple (*f* and *g*) and ultraviolet+yellow (*h*, *i*, and *j*) to a hummingbird eye. By our definition (see *Materials and Methods*), all of these colors are nonspectral, mostly stimulating two or three nonadjacent color cone types. Many natural reflectance spectra fall close to the nonspectral color axes and planes when represented in avian tetrahedral color space (*B*), which is here based on hummingbird vision. Using the TetraColorTubes, we generated colored lights corresponding to the numbered circles shown in avian color space. Our experiments, summarized here, reveal that hummingbirds can discriminate between pairs of colors—such as UV+red (dot 3) and pure UV (dot 1)—that fall on or near the UV+red, UV+green, and red+blue (purple) nonspectral axes (dashed lines). In addition, hummingbirds can discriminate between UV+yellow (dot 15) and yellow (dot 16). (*C*) Data from one of the experiments summarized in *B*. Here, hummingbirds were presented with two different UV+red colors, one a mixture of 75% UV and 22% red (rewarded with a sucrose solution; dot 2 in *B*) and the other a mixture of 23% UV and 71% red (not rewarded; dot 5 in *B*). In 10 trials, each comprising 25 "first choice" visits (*Materials and Methods*), we recorded whether birds visited the reward or nonreward color. To prevent birds from cueing on the position of the reward color, we swapped the positions of the reward and nonreward feeding stations at fixed intervals. The summary data are shown here (black dots), along with the number of visits expected (gray dots) if the colors are not discriminable. When the number of observed visits differed from the null expectation, we predicted that learning occurred: The birds could discriminate the two colors. We verified this with our Bayesian statistical model (see main text). Hummingbird image credit: Designed by Vexels.com. Tripod image credit: The Noun Project/Kari Gaynor, licensed under CC BY 3.0.

participated in each experiment (see additional details below). Overall, we conducted 19 experiments, summarized in Figs. 2 and 3 and described below, representing over 6,000 hummingbird visits. These included 6 validation experiments and 13 experiments on nonspectral colors (Figs. 2*B* and 3 and *SI Appendix*, Fig. S2 and Table S1).

To produce experimental stimuli, we designed a pair of TetraColorTubes. Each tube contained four LEDs (UV, blue, green, and red) whose intensities could be independently controlled to produce a wide range of colors in avian tetrahedral color space (Fig. 2*B* and *SI Appendix*, Fig. S1). This offered greater precision and flexibility than reflective stimuli. Inside the

REWARD WATER

Red v Green

Green v Red

UV$_{72}$+Red$_{26}$ v Red

UV$_{33}$+Green$_{44}$ v UV

UV$_{59}$+Green$_{27}$ v Green

UV$_{75}$+Red$_{22}$ v UV$_{23}$+Red$_{71}$

Purple v Red

Blue v Purple

UV$_{59}$+Green$_{27}$ v UV

UV$_{33}$+Green$_{44}$ v Green

UV$_{72}$+Red$_{26}$ v UV

Red v UV$_{25}$+Red$_{72}$

UV$_{33}$+Green$_{44}$ v UV$_{59}$+Green$_{27}$

Yellow v UV$_{22}$+Yellow

UV$_{13}$+Red$_{85}$ v Red

Null: Green v Green 2

Null: Green v Green 1

Null: Red v Red

Null: UV v UV

NONSPECTRAL

Euclidean distance in avian color space

1.00   0.50   0

0.5   0.6   0.7   0.8   0.9   1.0

Probability of choosing rewarded color

Fig. 3. Summary of color discrimination experiments in wild hummingbirds. Using a Bayesian statistical model (*Materials and Methods*), we estimated posterior densities for mean population color discrimination in each experiment, quantified as the per-visit probability of choosing the reward color across all experienced birds in the population (after controlling for feeder position bias). We assumed that birds were no worse than random at choosing the reward color. In experiments involving known distinguishable colors (red vs. green; green vs. red) and nonspectral colors, experienced birds chose the reward color substantially more often than chance, but in the control (null) experiments, the birds chose feeders quasirandomly (see main text). Lines (2.5%, 50%, and 97.5% quantiles) indicate the median and a 95% credible interval of the posterior distribution. The colored circles represent the stimuli presented in the discrimination experiments; note that the actual colors displayed were well mixed/uniform. The rewarded color is listed first and appears on the left. Experiments are ordered by decreasing Euclidean distance in avian color space (Fig. 2*B*) between stimuli. The blue shading of the densities is proportional to that distance.

tube, LEDs were housed behind a diffusing display surface so that colors produced by multiple LEDs (such as UV+red) would appear evenly mixed (*SI Appendix*, Fig. S1*B*). Colors were displayed at the end of the tube on a semitransparent circular acrylic surface (8.9 cm in diameter). A small nonreflective awning attached to the tube kept the light in shade. We quantified the output of the tubes using a spectrometer that was calibrated with a tungsten halogen light source. We then produced the desired stimuli by estimating how the hummingbird color cone types would be stimulated, using predicted hummingbird cone sensitivities (37) (Fig. 1*B*) (*Materials and Methods*). To control for possible effects of luminance differences, the stimuli were matched for double cone stimulation values for most experiments (*Materials and Methods*). This matching was done to help ensure that our experiments tested for color (hue/saturation) discrimination, not luminance discrimination.

In our experiments, we sampled from a large population of birds, only some of which were individually marked. Therefore, our objective was to estimate the hummingbird population's average capacity to discriminate reward colors in treatment and control experiments. To estimate this mean effect, we implemented a

Bayesian statistical model. We modeled the population as a mix of "experienced" birds (familiar with the current experiment and therefore predicted to visit the reward color preferentially if they can discriminate it) and "naive" birds (unfamiliar with the experiment and therefore expected to visit colors indiscriminately) (*Materials and Methods*). The relative frequencies of experienced and naive birds over time are not known a priori; they are inferred by the model. Our model estimates and adjusts for bias related to feeder position and experimental noise (*Materials and Methods*). Our measure of "mean population color discrimination" is the average per-visit probability of choosing the reward color across all experienced birds in the population (after accounting for position bias). Perfect discrimination by experienced birds would result in a mean population color discrimination value of 1, whereas indiscriminate visitation by experienced birds would give a value of 0.5 (*Materials and Methods*).

We carried out a series of validation experiments to ensure that our experimental design was appropriate. First, to verify that hummingbirds can quickly learn to discriminate colors that we expect to be easily discriminable (34), we showed that hummingbirds can discriminate red (reward) from green (nonreward) (Fig. 3 and *SI Appendix*, Fig. S2 and Table S1). Switching which color was associated with the reward gave a similar result (Fig. 3 and *SI Appendix*, Fig. S2 and Table S1). For these two experiments, our statistical model estimated that experienced birds tended to visit the rewarded color (Fig. 3). The mean population color discrimination was 0.79, with 99% posterior support for mean population color discrimination >0.65. Second, to ensure that our experiments were not capturing an unknown variable (e.g., odor, social learning), we performed a control (null) experiment, using green as the reward color and an identical green as the nonreward color. We found no evidence that hummingbirds could discriminate between identical colors (Fig. 3 and *SI Appendix*, Fig. S2 and Table S1). We repeated this control experiment each year (2016, 2017, and 2018) and in 2018 performed two additional control experiments (using identical UV and red lights, respectively). In these control experiments, our model estimated that experienced birds visited feeders without regard for color (Fig. 3). The mean population color discrimination was 0.58, with >99% posterior support for mean population color discrimination <0.67. These results strongly suggest that the frequent visits of experienced birds to reward colors in noncontrol experiments were indeed driven by color discrimination. Notably, the control experiments suggest that social learning, in which birds cue off of other birds' visitation behavior, is unlikely to explain the positive results of our color discrimination experiments, in which the mean population color discrimination was substantially higher than in the control experiments.

Having validated our experimental setup, we turned to nonspectral color discrimination. Typical color-matching experiments, in which an animal cannot distinguish a mixture of two monochromatic lights from an intermediate monochromatic light, are not possible with nonspectral colors. For example, in birds, visual models predict that there is no monochromatic light that will match a mixture of UV+green light. Instead, to investigate nonspectral color perception, we used an approach similar to that employed by Daumer (20), who performed extensive color vision experiments on bees. Honeybees are trichromatic, with one nonspectral color: UV+yellow [often called "bee-purple," and now technically known to be UV+green, since bees have UV-, blue-, and green-sensitive photoreceptors (38)]. Daumer demonstrated that bees can discriminate UV+yellow from its two components. He also showed that they can discriminate between different mixtures of UV and yellow light (20, 39). Using a similar rationale, we tested whether hummingbirds could distinguish between pairs of stimuli on (or near) the UV+red, UV+green, and purple nonspectral axes of avian tetrahedral color space (Fig. 2*B*), and

near the UV+yellow plane (representing ternary nonspectral colors) (Fig. 2*B*).

We found that broad-tailed hummingbirds can discriminate nonspectral colors throughout the avian tetrahedral color space (Figs. 2 *B* and *C* and 3 and *SI Appendix*, Fig. S2 and Table S1). On (or near) all three nonspectral axes, hummingbirds can discriminate between a nonspectral color and its "pure" components (falling on or near the monochromatic locus). For example, UV+red is distinct from both UV and red (Figs. 2*B* and 3 and *SI Appendix*, Fig. S2 and Table S1). In addition, hummingbirds can discriminate between two mixtures of nonspectral colors (Figs. 2*B* and 3 and *SI Appendix*, Fig. S2 and Table S1). For example, UV+green (with 33% UV) can be distinguished from another UV+green color (with 59% UV). The same is true for two different mixtures of UV+red (Fig. 2*C*). Finally, hummingbirds can discriminate a ternary nonspectral color, UV+yellow, from at least one of its component colors (yellow) (Figs. 2*B* and 3 and *SI Appendix*, Fig. S2 and Table S1), consistent with a previous finding in budgerigars (10).

In experiments involving nonspectral colors, our statistical model estimated that experienced birds visited the reward color substantially more often than chance (Fig. 3). The mean population color discrimination value across all nonspectral experiments was 0.83, with >99% posterior support for a mean discrimination >0.75. Posterior medians for population color discrimination in individual nonspectral experiments were all >0.71. In other words, our model estimated that experienced birds displayed a clear tendency to visit the reward color in all nonspectral experiments.

Our statistical model also enabled us to estimate whether birds were better at distinguishing nonspectral colors when the two colors were more different, as measured by Euclidean distance in avian color space (Fig. 2*B* and *SI Appendix*, Table S1). We observed increased (better) population color discrimination with increased Euclidean distance (more different) for colors near the UV+green axis, but no clear trend for the UV+red axis (*SI Appendix*, Fig. S3). How animals perceive differences between suprathreshold (widely separated in color space) colors is largely unknown (24). The observed difference between the UV+green and UV+red axes suggests that suprathreshold color perception may not be uniform throughout the avian color space, a topic we can explore more fully in the future using this experimental system.

As described above, our statistical model estimated the average discrimination capacity across all experienced birds in the population. We used a population-level measure because some individual birds may fail to learn to discriminate colors, even when discrimination is possible for other individuals (5), and because population average discrimination rates are often more ecologically important than any individual rate (e.g., for determining overall rates at which discriminable flower species will be pollinated). Color discrimination at the population level in all of the nonspectral experiments indicates strongly that at least some hummingbirds are individually capable of discriminating nonspectral colors. Our population-level approach means that we do not know exactly how many individuals visit the feeders, nor do we know the underlying distribution of individual color discrimination ability. However, it is unlikely that the high estimated average discrimination capacity we observe is due to a small number of extremely accurate individual experienced birds (see *Materials and Methods*); many birds in our population are likely discriminating nonspectral colors. Nevertheless, uncovering variation in nonspectral color discrimination among individual hummingbirds in our study population is a goal for future research.

Investigating Nonspectral Colors in the Natural Environment

A common approach to analyzing colors in a way that is relevant to animal vision involves modeling reflectance data in a color space (or chromaticity diagram) defined by the number and sensitivities of an animal's photoreceptors (5) (Fig. 1*A*). Many previous studies have used color spaces to explore the relationship between visual signals and the visual systems of birds and other animals (40–44). Importantly, these color spaces are models. Behavioral experiments on color discrimination—including those we conducted in this study—are attempts to understand and validate these color space models (5).

Having shown that hummingbirds can discriminate a variety of nonspectral colors, we next investigated the extent to which nonspectral color perception may be ecologically important in the wild. In a color space based on a model of hummingbird color vision (37), we analyzed colors in two large datasets: 965 bird plumage spectra (42) and 2,350 plant spectra (42, 45). Our analysis showed that ~30% of these plumage colors and ~35% of these plant colors would be perceived as secondary and/or ternary nonspectral (Fig. 4). Purple (SWS+LWS) and UV+purple (UVS+SWS+LWS) nonspectral colors are much more common in plants than in plumage (Fig. 4), perhaps due to anthocyanin pigments, which plants produce but birds cannot synthesize in their feathers (42). A comparable analysis of the same data using a model of human vision (cone sensitivity curves from ref. 46) revealed that humans would perceive only 7% of the plumage colors and 19% of the plant colors as nonspectral. In a separate study on bees, a similar analysis in a hexagonal honeybee color space showed that roughly 12% of flower colors would fall in a region corresponding to the bee nonspectral color UV+green (44).

Discussion

Our experiments demonstrate that hummingbirds can discriminate colors on (or near) all secondary nonspectral axes and one ternary nonspectral color plane in avian tetrahedral color space. These results are consistent with the claim that birds are tetrachromats, such that the avian color space (Fig. 1*A*) represents a vast range of behaviorally and ecologically relevant colors, many of which humans (or any trichromat) cannot even imagine (4, 47, 48). Showing that birds can discriminate a variety of nonspectral colors provides a step forward in our understanding of the dimensionality of bird vision. It suggests (but does not prove, see below) that interactions involving nonadjacent cone types contribute to color vision in birds, providing a rich tetrachromatic experience. Although these experiments were performed with hummingbirds, our findings are likely relevant to all diurnal, tetrachromatic birds and probably to many fish, reptiles, and invertebrates.

What are the neural mechanisms responsible for nonspectral color vision? Although our experiments cannot reveal the mechanistic basis for hummingbirds' nonspectral color discrimination, we can make inferences about neural comparisons. Postreceptor neural comparison of photoreceptor outputs (i.e., it is not the absolute stimulation of the photoreceptors in isolation that matters but rather their relative stimulation) is a fundamental principle underlying most models of color vision (but see ref. 49 for a discussion of alternatives). These models include chromaticity diagrams (like the avian tetrahedral color space), which encode spectra as a function of how they would relatively stimulate the color cone types (5). Chromaticity diagrams assume—but do not specify—some kind of relative comparison. By providing support for the avian tetrahedral color space, our findings suggest some kind of comparison of the color cone type outputs. How might this comparison be achieved?

There is widespread support for the notion that neural comparisons are made possible by opponent processing, in which the outputs of different photoreceptor classes are compared antagonistically by neurons with inhibitory and excitatory responses to different photoreceptor signals (2, 14, 24, 50). Opponent processing appears to be a key ingredient for color vision in most animals (24, 51, 52) and is probably operating in birds (reviewed in ref. 2), but we still know staggeringly little about the neural coding of color in the avian visual pathway (53). Although we lack knowledge of the precise (potential) opponent channels in birds (54), some clues may come from homologous tetrachromatic
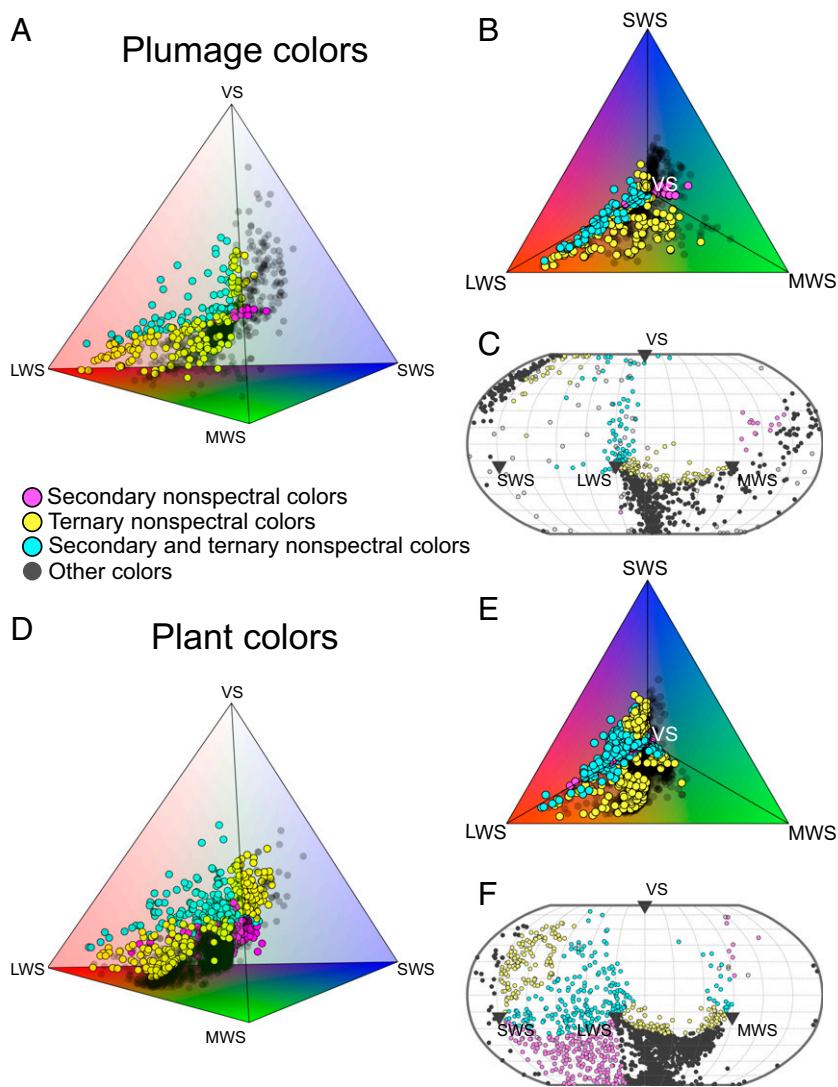
Fig. 4. Nonspectral colors make up a large proportion of the avian visual world. (*A*) Diverse plumage colors (*n* = 965) are shown in hummingbird color space. The distribution is also shown as if looking down from the tetrahedron's apex (the VS cone) (*B*) and as a Robinson projection (*C*), which illustrates variation in hue (*Materials and Methods*). We estimated that ~30% of these colors would be perceived by hummingbirds as secondary (magenta dots), ternary (yellow dots), or both secondary and ternary (turquoise dots) nonspectral. We estimated that just 7% of these colors would appear nonspectral to humans. (*D*–*F*) A comparable analysis of diverse plant colors (*n* = 2,350) revealed that hummingbirds would likely perceive ~35% of these colors as nonspectral, compared to just 19% by humans. Purple (stimulating the SWS and LWS cones) and UV+purple (stimulating the VS, SWS, and LWS cones) nonspectral colors are much more common in plants than in plumage (see main text). The black dots represent colors that are not nonspectral. In the Robinson projections (*C* and *F*), colors that are close to achromatic (white/gray) are shown in light gray (*Materials and Methods*).

turtles (55): Neural recordings suggest the presence of at least five color-opponent channels (mediated by ganglion cells), several of which correspond to nonspectral colors. Some color-opponent cells have also been reported in the pigeon thalamus (56). We expect that additional cells will ultimately be found in birds.

Support for some kind of postreceptor neural comparison in birds comes from our experiment showing that hummingbirds can discriminate a mix of 33% UV plus 44% green from a mix of 59% UV plus 27% green. In theory, birds might distinguish these colors based on differences in absolute stimulation of the MWS (green) cone alone, without requiring comparison to the UVS/VS cone, or vice versa. However, in this experiment, we estimate that the absolute stimulation of the MWS cones for both color mixes was very similar. Therefore, information from the MWS cone alone would not likely permit birds to discriminate these colors. The absolute stimulation of the UVS/VS cones was different for the color mixes, but we suspect that broad-tailed hummingbirds do not

discriminate pure UV colors on the basis of intensity (brightness) (36). Therefore, it is highly plausible that the birds possess some postreceptor neural mechanism for comparing the relative stimulation of the UV- and green-sensitive cones, which allowed them to discriminate between the two different mixtures of light. A similar inference can be made from our experiment showing that birds can discriminate between two different mixtures of UV+red.

Overall, the results of our experiments are consistent with (but do not prove) the idea that birds possess a tetrachromatic visual system that involves comparisons of adjacent and nonadjacent cone types. This color vision system can be modeled in a tetrahedral color space (Fig. 1*A*) in which birds can discriminate a broad range of colors, including nonspectral colors. In the future, strong support for tetrachromacy could come from a more direct test of dimensionality: color-mixing experiments showing that four monochromatic lights (primaries) are necessary and sufficient for creating any bird-perceived color, including broadband

"white" light (2, 14). Color-mixing experiments provided evidence for tetrachromatic color vision in goldfish (57), but comparable experiments have been attempted rarely in birds (58).

Even if the neural mechanisms for color vision were clear, and even if color-mixing experiments attest to avian tetrachromacy, we still could not answer the more philosophical question of what nonspectral colors really look like to birds. Does UV+green appear to birds as a mix of those colors (analogous to a double-stop chord played by a violinist) or as a sublime new color (analogous to a completely new tone unlike its components)? We cannot say. Even in humans, where we have language to describe our experiences, we are a long way from understanding what constitutes "unique hues" (not composed of other colors) (59), and evidence that nonhuman animals possess unique hues is lacking (23). Ultimately, what matters to a bird is probably not whether colorful signals are detected by adjacent or nonadjacent cone types: It is how those colors function to provide information about food, mates, or predators.

A final caveat is that we have assumed that hummingbirds, like almost all other diurnal birds studied to date (7), possess the typical complement of four color cone types. This inference is supported by several lines of inquiry, including behavioral data on hummingbird color discrimination (36), models derived from electroretinography (60, 61), microspectrophotometry (62), and genetic analyses (37) (see additional details in *Materials and Methods*). Nevertheless, a recent analysis failed to identify the *sws1* visual pigment opsin gene (associated with the UVS/VS cone type) in the genomes of the Anna's hummingbird (*Calypte anna*) and the closely related chimney swift (*Chaetura pelagica*) (63). Hummingbirds and swifts form a diurnal subclade nested in the historically nocturnal Strisores, a group that includes nightjars, nighthawks, and relatives (64). We cannot preclude the possibility that the opsin genes in hummingbirds and swifts have evolved divergently, influenced by their nocturnal ancestry, potentially leading to an unconventional color vision system. Opsin gene loss or modification might be more common than previously considered: Transcriptomic and genomic analyses have uncovered several instances of opsin gene loss in birds (reviewed in ref. 53), including widespread *sws1* loss in owls (65, 66). Surprisingly, *sws1* appears to be present in two nightjar species, chuck-will's-widow (*Antrostomus carolinensis*) (63) and European nightjar (*Caprimulgus europaeus*) (67). This raises the interesting question of when *sws1* was lost, if indeed it is absent in hummingbirds. Although some opsin genes seem to be particularly challenging to sequence from the genome (63, 68), the time is ripe for a detailed analysis of opsin gene evolution and expression in Strisores.

Our experiments show that broad-tailed hummingbirds can detect UV light and distinguish a range of nonspectral colors that include UV. If hummingbirds lack a UV-sensitive cone type and functional *sws1* gene and/or other opsin genes, it will be fascinating to consider alternative color vision mechanisms that could explain these results. A more limited suite of cone opsins could compensate for the loss of *sws1* by being tuned to different wavelengths. A visual pigment's sensitivity can be tuned in many ways (reviewed in ref. 52), including spectral filtering by ocular media and oil droplets (69). Not only could a non-*sws1* visual pigment be tuned to recover some UV sensitivity, in theory a single pigment—if variably modified/filtered in the same eye—could provide an extra dimension of color vision (2). Remarkably, this may occur in a *Heliconius* butterfly that can discriminate different red colors using a single LWS opsin, the sensitivity of which is modified by one of two lateral filtering pigments (70). In theory, a similar mechanism could boost a bird with just three cone opsins to functional tetrachromacy. In addition, the double cones and/or rods might contribute to color vision in ways we have not yet appreciated. Moving forward, it will be essential to perform comprehensive microspectrophotometry, retinal transcriptomics, and genomic analyses to confirm the physiological and genetic bases of color vision in hummingbirds.

However birds perceive nonspectral colors, these colors are prevalent in their natural environments. Our analysis of plumage and plant colors shows that—for tetrachromatic animals—nonspectral colors are likely important for communication and foraging. We do not suggest that nonspectral colors are more important than other colors in the environment, or that nonspectral color perception is special for birds: Their capacity to discriminate many nonspectral colors is a consequence of a four-cone color system. However, nonspectral color perception has received little formal research attention, in part because humans lack many nonspectral colors likely perceived by other animals. Because early vertebrates possessed four color cone photopigments, an ancient condition present in all major branches of vertebrates except mammals (21), tetrachromacy—and the rich perception of nonspectral colors it affords—is likely widespread. Investigating how nonspectral color perception may shape signal evolution and diversity across birds and other vertebrate taxa is an exciting and timely prospect, with many potential new lines of inquiry.

First, how are nonspectral colors used for signaling? Nonspectral plumage and plant colors colonize somewhat different regions of avian color space (Fig. 4). For plumage, nonspectral colors primarily occupy the UV+red, UV+green, and UV+yellow regions of color space, whereas for plants nonspectral colors largely occupy the purple and UV+purple regions (and UV+red and UV+yellow, to a lesser degree). Perhaps the rarity of purple colors in plumage makes it a particularly salient color for flower pollination. A recent analysis shows that competition for hummingbird pollination has led to explosive diversity in flower color—especially purple—in a radiation of Andean shrubs (*Iochrominae*) (71). Alternatively, perhaps purple plumage is reserved for specific signaling contexts: The magenta gorget of male broad-tailed hummingbirds, which features prominently in a spectacular multimodal courtship dive (72), is likely perceived as a nonspectral color by birds (Figs. 1D and 2 A, f).

Second, what physical mechanisms give rise to nonspectral colors? In plants, many purple colors arise from anthocyanin pigments, which birds break down during digestion and cannot synthesize, leading to a paucity of purple plumage colors (42). In plumage, many nonspectral UV+red and UV+green colors likely arise from the combination of structural color and dietary carotenoid pigments (42), but some nonspectral colors—like the belly of the paradise tanager (*Tangara chilensis*) (Fig. 2 A, d)— are purely structural. Other vivid nonspectral colors are produced by diverse pigmentary mechanisms. Examples include the following: the UV+yellow wing of the fire-maned bowerbird (*Sericulus bakeri*) (Fig. 2 A, h), made by a carotenoid pigment; the UV+red crown of the Papuan lorikeet (*Charmosyna papou*) (Fig. 2 A, b), made by a psittacofulvin pigment; and the UV+red patch on the wing of the Hartlaub's turaco (*Tauraco hartlaubi*), made by a turacin pigment. A macroevolutionary analysis of nonspectral colors in plumage and plants could reveal the selective pressures to which these colors are most subjected.

Finally, because our experiments were performed on wild, free-flying animals, they demonstrate not only that birds can discriminate nonspectral colors but also that they do in the wild, providing what psychologists call "ecological validity" (73): the capacity to generalize to the real world. We have shown that these "wild psychophysics" experiments—minimally invasive and requiring no extensive training—can be a powerful tool for investigating real-world color perception, even if they may offer less precise control than the laboratory. Moving forward, this system can be used to evaluate fundamental predictions about animal color vision—from color discrimination (9) to higher-order processes like categorical perception (74)—in the wild, providing a critical field test of classic models (5, 24) in visual ecology.

Materials and Methods

Conducting Field Experiments. We conducted fieldwork near RMBL in Gothic, Colorado, in June of 2016, 2017, and 2018. At this site, broad-tailed hummingbirds are the only resident breeding hummingbirds. Males migrate to Colorado in late spring from their wintering grounds in Mexico and Central America, followed by females. Broad-tailed hummingbirds forage for nectar from a variety of plant species that bloom throughout the summer (75). Early in the breeding season, in May and June, males and females will frequently visit feeders (76), while floral abundance is still relatively low. Fieldwork was conducted with permission from RMBL and approved by the RMBL Animal Care Committee.

Experimental Design. We performed color discrimination experiments (see main text for additional details) by training hummingbirds to associate one color produced by a TETRACOLORTUBE (reward color) with an ~35% (w/v) sucrose solution and a second tube color (nonreward color) with water. Each experiment consisted of multiple trials. Trial lengths were 25 visits (2016 and 2017) or 15 min (2018); for details, see *SI Appendix*: "Trial length." We swapped the positions of the reward and nonreward setups at the end of each trial to prevent hummingbirds from learning spatial cues. When a hummingbird arrived at the experimental setup, an observer recorded whether it first visited the reward or nonreward color (Fig. 2C and *SI Appendix*, Fig. S2; see main text for details). We performed the experiments in an open field near a small wooden building, the exterior wall of which formed the end of the experimental arena. The tripods on which the tubes were mounted were positioned in front of the wall with the tubes facing out to the field, at a height of 1.4 m. This placement prevented birds from approaching the feeders from behind, thereby ensuring that they viewed the tubes.

In our experiments, we sampled from a large, initially untrained population of birds; we did not know the number or individual identities of the birds. Therefore, we estimated the hummingbird population's average capacity to discriminate reward colors in experiments. Rather than performing an initial training period (the data from which might be subsequently excluded), we included all primary visits during the experiment in our analyses. We did this because we expect that there is considerable turnover of birds visiting the experimental setup over the course of the day, making it unreasonable to restrict training to a few hours in the morning (for example). If we detect a large population average discrimination capacity, this is despite noise arising from visits by birds that 1) are still becoming trained/experienced; 2) are moving transiently through the field site; 3) fail to learn; or 4) adopt an alternative choice strategy. More details are provided below in "Bayesian modeling." See also main text and *SI Appendix*: "Additional details: experimental design."

Designing and Calibrating the TETRACOLORTUBES. In order to produce visual stimuli, we designed a pair of light-emitting devices: the TETRACOLORTUBES. Each tube contained four high-brightness LEDs with minimally overlapping emission spectra (*SI Appendix*, Fig. S1). Peak spectral outputs (in microwatts per square centimeter per nanometer) were centered at 365, 457, 525, and 623 nm (LED Engin, Inc.: LZ1-10U600-0000, LZ1-10B202-0000, LZ1-10G102-0000, and LZ1-10R102-0000). Note that our calculations using bird visual models were performed after light measurements were converted to quanta (see *SI Appendix*: "Additional details: producing stimuli for field experiments"). The LED emission spectra do not align perfectly with the predicted peak sensitivities of a hummingbird's four color cones, but they cover much of the bird-visible spectrum (*SI Appendix*, Fig. S1A). Consequently, a large portion of the avian tetrahedral color space (of theoretically visible colors) can be produced by a TETRACOLORTUBE (Fig. 2B).

Each tube consisted of a custom-machined cylindrical aluminum enclosure, which ensured proper positioning and heat sinking of the diodes (*SI Appendix*, Fig. S1B). LEDs were mounted so that their projected light cones overlapped before being scattered by a highly diffusing display surface (abraded UV-transmitting acrylic plastic). Regions where the light cones did not overlap were visually occluded by an opaque annular mask. Customized electronics, housed within a separate enclosure, provided drive currents to the LEDs and a USB interface to the field computer. The intensity of each color channel was controlled via pulse-width-modulation (8-bit resolution: 256 levels of duty-cycle) of a 1-A current at 400 Hz. Note that 400 Hz is well above the recorded flicker fusion rates of birds (77), so we expect that hummingbirds observed visual stimuli produced by the tubes as static and nonflickering. A simple user interface (LabView, National Instruments Corporation; or MATLAB, MathWorks) communicated with the tubes and allowed for in-field modulation of color and brightness, so that we could produce stimuli for field experiments.

The absolute irradiance of light emitted from the TETRACOLORTUBES was measured using an OceanOptics USB4000 spectrometer with an OceanOptics CC-3-UV-S cosine corrector, which was held flush to the tube's exterior diffuser. Before obtaining absolute irradiance measurements from the tubes, we calibrated the spectrometer using a standard light source (OceanOptics; HL-3-CAL). Radiance, which differs from irradiance in that it captures flux density over a solid viewing angle, is often measured. Here, measurements using irradiance and radiance yielded similar results (see *SI Appendix*: "Additional details: producing stimuli for field experiments").

Producing Stimuli for Field Experiments. Our objective was to generate colored stimuli throughout the avian tetrahedral color space (Fig. 2B), especially on or near the nonspectral color axes (Fig. 1A). Briefly, our process involved the following: 1) Choosing a target color (for example, 15% UV plus 85% red; Fig. 2B, dot 6). This color has a precise location (in this case, on the UV+red axis) in an avian color space based on hummingbird vision. 2) Determining the duty cycles of the TETRACOLORTUBE LEDs that would produce the target color (i.e., a color that would stimulate the hummingbird eye in the desired way, with relative color cone stimulation values {VS, SWS, MWS, LWS} ~ {0.15, ~0, ~0, 0.85}). To estimate the relative cone stimulation values, we used predicted hummingbird cone sensitivities (37), custom code, and functions in the R package "pavo" (78). 3) Measuring the produced color (the absolute irradiance of the tube) using a spectrometer and verifying that the estimated color cone stimulation values (*SI Appendix*, Table S1) were close to those of the target color. We explain this process in more detail in *SI Appendix*: "Additional details: producing stimuli for field experiments."

Hummingbird Color Vision. Hummingbirds are presumed to have four color cone types. This inference comes from behavioral data on hummingbird color discrimination (36), models derived from electroretinography (60, 61), microspectrophotometry (MSP) (62), and genetic analyses (37). For example, MSP of blue-throated hummingbirds (*Lampornis clemenciae*) revealed the presence of the five typical oil droplet types found in birds, including the transparent type usually found in the UVS/VS cone type (62). In general, each oil droplet type is associated with a specific cone photoreceptor (i.e., the four single color cone types and the double cone) in birds (7, 79). Their presence in hummingbirds therefore hints at a four-color cone-type system, although the MSP analysis (62) did not explicitly link oil droplets to their cone types and visual pigments. The presence of a UV-sensitive cone type specifically is supported by electroretinography (6, 60), behavioral experiments in several hummingbird species (35, 80)—including broad-tailed hummingbirds in the present study, and the identification of the *sws1* visual pigment opsin gene (associated with the UVS/VS cone type) via DNA sequencing in three distant hummingbird clades (37) (discussed below).

Diurnal birds typically possess four color cone types (7), but—across species—they differ in whether the fourth color cone type contains UVS or VS *sws1* opsin pigments. Both the UVS and VS cone types are broadly sensitive to UV wavelengths (<400 nm); the key difference is that the VS cone type has a maximum sensitivity between 402 and 426 nm, while the UVS cone type has a maximum sensitivity between 355 and 380 nm (81). Whether the broad-tailed hummingbird has the UVS or VS cone type is unknown. However, sequencing of the *sws1* opsin gene in three Neotropical hummingbird species suggests that hummingbirds possess the VS cone type (37), an inference supported by a broad phylogenetic reconstruction of *sws1* opsin evolution (81). This molecular evidence seems to conflict with physiological results suggesting that the UVS cone type is present in the ruby-throated hummingbird (*Archilochus colubris*) (6, 82) and the green-backed firecrown (*Sephanoides sephaniodes*) (60). One possible explanation is that hummingbirds do possess a VS cone type, but it is tuned toward the shorter-wavelength (toward UV) end of the VS range. This might provide hummingbirds with greater UV sensitivity, which is further facilitated by a UV-transparent ocular medium (37). Given this, we assume a VS system for hummingbirds, using spectral sensitivity curves reported in Fig. 1B from ref. 37. However, to demonstrate that our results about nonspectral color perception would be robust to a UVS visual system, we also estimated cone stimulation (see "Additional details: producing stimuli for field experiments" in *SI Appendix*) for all experimental stimuli using an average UVS visual system and double cones for European starling (*Sturnus vulgaris*), a UVS species. This analysis indicated that all nonspectral color stimuli in our experiments would be perceived as nonspectral by either a VS or a UVS bird.

We used double cone sensitivity curves for the chicken (*Gallus gallus domesticus*), which has VS vision, to estimate the perceived luminance of color stimuli used in experiments. The double cones are thought to mediate luminance-based tasks in birds (83, 84). For 16 of the 19 experiments, stimuli were balanced for similar luminance, with Michelson contrasts less than ~4% (85), with the exception of the red/green validation experiments, where Michelson contrasts were less than ~10%. Note: in a subsequent field season, we repeated the red vs. green validation experiment with Michelson

contrasts <4% and showed that birds could still discriminate these colors. For consistency, we report results from the original 2017 experiments. Moreover, a ~10% Michelson contrast appears to be below the brightness discrimination threshold for some birds (86). For the remaining three experiments (marked with an asterisk in *SI Appendix*, Table S1), it was not possible to create luminance-balanced stimuli due to the very low double cone sensitivity to UV light. This is a physiological reality of the cone types rather than a technical limitation. In these experiments, stimuli were instead balanced for approximately equal absolute UV (VS cone type) stimulation.

Bayesian Modeling. To estimate the extent to which hummingbirds learned to discriminate between two colors, we designed a Bayesian probabilistic model. Because we initiated data recording immediately (i.e., without an initial training period), we assumed that observed visits to a feeder are attributable either to birds that are "experienced" (regarding the current pair of reward and nonreward colors) or "naive." We assumed that the ratio of visits attributable to experienced versus naive birds increases over the course of the experiment due to naive birds becoming experienced, but eventually it saturates due to experienced birds leaving and being replaced by new naive birds (*SI Appendix*, Fig. S4). We assumed that naive birds visit the reward color and the nonreward color with equal probability (0.5 each), modified in a given trial by an unknown population bias toward either the left or right feeder (irrespective of current reward location). Experienced birds visit in the same way, but with some additional unknown tendency to choose the reward color: an experience effect. We explain the model in detail in *SI Appendix*: "Description of the Bayesian model." In the main text, we report the mean population color discrimination for each experiment (Fig. 3). This is the estimated average probability of a correct choice across all visits attributed to experienced birds, after accounting for positional preference (bias) and noise. Perfect discrimination by experienced birds would result in a value of 1, whereas indiscriminate visitation by experienced birds would give a value of 0.5.

Note that our definition of "experienced" will only apply to birds who undertake an accurate foraging strategy of preferentially visiting the rewarded color (that is, they choose it more often than chance, provided they can distinguish it from the unrewarded color). Birds that have learned the experiment but adopt a different foraging strategy (perhaps they are speedy instead of accurate, preferring instead to visit both the rewarded and unrewarded colors in rapid succession, without attending to color cues) are indistinguishable from truly naive birds in our data and our modeling. In many animal populations, some individuals might be accurate in discrimination tests, while others are speedy (87). In our experiments, evidence of population-level learning—despite experimental noise introduced by both truly naive birds and those using alternative strategies—indicates that some birds do indeed discriminate the colors and use an accurate approach. In other words, our positive results are conservative. Investigating the extent to which individuals use alternative strategies is a future research aim.

Note that even in the control (null) experiments, our model estimates that some proportion of visits is attributable to "experienced" birds. This is because when the model estimates that birds cannot discriminate colors and the additive probability of being correct when experienced (the experience effect) is negligible, naive and experienced birds should both visit feeders regardless of which feeder possesses the reward. The mean population color discrimination should in this case approach 0.5 (indiscriminate visitation).

Defining Nonspectral Colors. Two definitions of nonspectral are present in the literature. In the broad definition, a nonspectral color is any color that does not lie along the monochromatic locus (i.e., the colored line in Fig. 1A). For birds, this definition would include as nonspectral any color that stimulates more than two cones because such a color could not be evoked by a single monochromatic light and thus does not fall on the monochromatic locus/spectral line. The narrower definition (3, 4, 13, 15, 16), and the one we employ here, places emphasis not on the properties of light (i.e., monochromatic or not) but on the stimulation of the cones: In this case, nonspectral colors are defined as those that primarily stimulate nonadjacent color cone types. For a tetrachromat, secondary nonspectral colors involve two nonadjacent cone types, and ternary nonspectral colors involve three (two adjacent cone types, both of which are nonadjacent to a third) (15, 16). To enable quantification of nonspectral colors, we adopt a general quantitative definition of a nonspectral color, which can be applied to tetrachromatic (and other) animals. For a given color, we calculate the relative stimulation of each of the color cone types (here using a model of hummingbird color vision). We then rank the cones from highest to lowest stimulation, excluding any cones that have less than 1% relative cone stimulation (which could represent measurement error/noise). If the two highest cones are nonadjacent, then the color is secondary nonspectral. If the top three cones are nonadjacent (meaning two are adjacent to each other but neither is adjacent to the third cone), then the color is ternary nonspectral. A given color can be secondary nonspectral, ternary nonspectral, both secondary and ternary nonspectral, or none of these.

Our definition is a simple starting point, and more stringent rules could be applied. For example, one could require that for a color to be secondary nonspectral, the nonadjacent cone stimulations must be much higher (i.e., above some threshold) than the two other cone stimulations. Another measure of the strength of nonspectrality could be proximity (inverse Euclidean distance) to the midpoint of a nonspectral axis or the center of a nonspectral plane. An additional caveat is as follows: Our current definition would consider a color that is close to achromatic (gray) to be nonspectral if the two (or for ternary, three) most stimulated cones are nonadjacent, even if the difference across cones is very slight. A color that is {UVS SWS MWS LWS} = {0.26 0.24 0.26 0.24} would be considered a UV+green secondary nonspectral color. Additionally, our definition would not characterize pure white or gray or black as nonspectral, since in these cases the stimulation of the four cones is equal. However, white and gray are sometimes considered to be a special class of nonspectral colors, since they are not found in the rainbow, cannot be produced from monochromatic light, and require stimulation of nonadjacent cones. In Fig. 4, alongside the plots of plumage and plant colors in avian tetrahedral space, we show Robinson projections (C and F), which illustrate variation in hue (88). Colors that are close to achromatic (with SD among the four cone stimulation values <0.015) are shaded light gray on the projections.

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