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COMMENTARY

Avian color expression and perception: is there a carotenoid link?

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ABSTRACT

Carotenoids color many of the red, orange and yellow ornaments of birds and also shape avian vision. The carotenoid-pigmented oil droplets in cone photoreceptors filter incoming light and are predicted to aid in color discrimination. Carotenoid use in both avian coloration and color vision raises an intriguing question: is the evolution of visual signals and signal perception linked through these pigments? Here, we explore the genetic, physiological and functional connections between these traits. Carotenoid color and droplet pigmentation share common mechanisms of metabolic conversion and are both affected by diet and immune system challenges. Yet, the time scale and magnitude of these effects differ greatly between plumage and the visual system. Recent observations suggest a link between retinal carotenoid levels and color discrimination performance, but the mechanisms underlying these associations remain unclear. Therefore, we performed a modeling exercise to ask whether and how changes in droplet carotenoid content could alter the perception of carotenoid-based plumage. This exercise revealed that changing oil droplet carotenoid concentration does not substantially affect the discrimination of carotenoid-based colors, but might change how reliably a receiver can predict the carotenoid content of an ornament. These findings suggest that, if present, a carotenoid link between signal and perception is subtle. Deconstructing this relationship will require a deeper understanding of avian visual perception and the mechanisms of color production. We highlight several areas where we see opportunities to gain new insights, including comparative genomic studies of shared mechanisms of carotenoid processing and alternative approaches to investigating color vision.

KEY WORDS: Avian color vision, Oil droplets, Carotenoid-based plumage, RNL modeling

Introduction

The elaborate coloration of animals, especially birds, is a striking example of biological diversity, and these traits are a model system for the study of evolution (Cuthill et al., 2017; Hill and McGraw, 2006). The complexity of bird colors raises questions of how and why these traits have evolved. Substantial evidence implicates sexual selection as a major evolutionary driver; for example, female preference can result in elaborately colored males enjoying greater reproductive success than their drabber counterparts (Hill, 2006). A variety of models have been developed to explain the evolution of sexually selected traits. A common element of many of these models is the co-evolution of trait expression and preference for the trait. For instance, the 'sensory drive' model predicts that perceptual biases arise from environmental conditions that, in turn, shape preferences and the evolution of signals such as coloration (Endler, 1992; Price, 2017). 'Fisherian runaway' models predict that genetic linkage between trait and preference establishes a positive covariance leading to the rapid and extreme elaboration of both trait and preference (Fisher, 1930; Xu and Shaw, 2019). Thus, resolving the evolution of sexually selected traits requires an understanding of the physiology and genetics of three interacting components: (1) the traits under selection, (2) the preference for those traits and (3) the sensory systems that mediate trait assessment.

Carotenoids are the pigments that produce the brilliant red, orange and yellow coloration of many bird species (Blount and McGraw, 2008). These traits play important roles in mate choice, and their expression has been linked to aspects of individual quality (Hill, 2006, 2007; Svensson and Wong, 2011; Weaver et al., 2018). Carotenoid-based colors are now considered a model system for investigating the evolution of elaborate visual displays. Although best known for their role in signaling displays, carotenoids also play an essential role in avian vision. Carotenoid-pigmented oil droplets within the cone photoreceptors (see Glossary) function as spectral filters (see Glossary) that are predicted to fine-tune the sensitivities of the avian eye, facilitating color vision, but also limiting absolute sensitivity (Box 1; Goldsmith et al., 1984; Toomey et al., 2015; Vorobyev et al., 1998). Thus, carotenoids might influence the way a bird sees the world, including the perception of carotenoid-based visual signals. This raises the intriguing possibility that both the production and perception of carotenoid-based visual signals might be shaped by shared environmental, physiological and genetic factors.

In this Commentary, we will focus on recent developments in our understanding of the biochemistry, physiology, genetics and function of carotenoids in birds. We do this through a literature synthesis and a modeling exercise to examine how changes in oil droplet carotenoid content might alter the perception of carotenoid-based plumage. Although we focus on birds, carotenoid-based coloration is widespread amongst vertebrates (Blount and McGraw, 2008), and carotenoid-based spectral filtering is an important component of the visual systems of many taxa, including reptiles, amphibians and fish (Toomey and Corbo, 2017). Therefore, there are opportunities to apply the ideas discussed here beyond birds; indeed, non-avian taxa may offer unique insights, especially those with distinct visual ecologies.

The carotenoid chemistry of avian color and vision

Carotenoids are among the most abundant pigments in the natural world; these tetraterpenoid molecules (see Glossary) are an important part of the light-harvesting apparatus of photosynthetic organisms (Britton et al., 2008; Goodwin, 1984). Birds cannot produce carotenoids *de novo* and must acquire them through their diet (McGraw, 2006). The diets of terrestrial birds typically contain just a few carotenoid types; the most abundant among these are lutein, zeaxanthin and β-carotene (McGraw, 2006). These typically impart yellow to orange colors, yet avian coloration extends well beyond this limited palette through substantial metabolic

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Glossary

Achromatic contrast
A measure of the difference in the intensity or luminance between two visual stimuli. Similar to chromatic contrast models, achromatic contrasts can be scaled to receptor noise and take into account the irradiance properties of the ambient light and the visual parameters of the receiver.

Apocarotenoid
Cleavage products of carotenoids usually containing fewer than the 40 carbon atoms of the parent compound (e.g. galloxanthin).

Chromatic contrast
A measure of the difference in the spectral composition of two visual stimuli irrespective of their intensity (i.e. brightness). Chromatic contrast models calculate this as a distance in color space, relative to receptor noise, while taking into account the ambient light and visual parameters of the receiver.

Color space
A graphical construct where visual stimuli are represented as points in a multi-dimensional space determined by the relative stimulation of the photoreceptors mediating color vision. Each node in color space represents a different cone photoreceptor; therefore, trichromatic humans have a triangular-shaped color space with three nodes. In contrast, tetrachromatic animals such as birds have four single cones that contribute to their tetrahedral color space.

Cone oil droplet
Specialized organelle of the cone photoreceptors in some vertebrate animal groups (including birds, reptiles and amphibians); these function as optical elements and spectral filters if pigmented.

Cone photoreceptor
Specialized light-sensitive cell of the vertebrate retina. Cones are generally less sensitive than rod photoreceptors, but respond more rapidly and function best in bright environments. In birds, the single cone photoreceptors mediate color vision, whereas the double cone is thought to mediate motion detection or achromatic vision.

Ketocarotenoid
A carotenoid that contains a ketone group in its β-ionone ring. Here, we are primarily referring to C4-ketocarotenoids with the carbonyl at the 4 and/or 4′ carbon of the molecule.

Optical density
A measure of absorbance calculated by the logarithmic ratio of light intensity on a material to the intensity of transmitted light.

Quantum catch
The quantity of incident photons captured by visual pigments of a photoreceptor.

Receptor noise
Fluctuations in photoreceptor signaling resulting from spontaneous activation of the phototransduction cascade in the absence of light and the stochastic nature of photon absorption. This noise sets a lower limit on the stimulus magnitude necessary for discrimination (i.e. resolving chromatic/achromatic contrasts). Receptor noise-limited (RNL) visual models take estimates of receptor noise into account when predicting the just-noticeable difference between two visual stimuli.

Spectral filter
A structure that selectively transmits specific wavelengths of light. In avian vision, the carotenoid-pigmented oil droplets prevent particular wavelengths from reaching the visual pigment, thus acting as spectral filters.

Tetraterpenoid molecules
Hydrocarbons consisting of eight isoprene units (C_5H_8) that may also have oxygen-containing functional groups.

transformations (LaFountain et al., 2015; Morrison and Badyaev, 2016). It is estimated that >29% of bird species have at least one patch of carotenoid-pigmented feathers, and that carotenoid-pigmented bare parts (e.g. skin, beaks and legs) may be even more common (Iverson and Karubian, 2017; Thomas et al., 2014). So far, 39 different carotenoid types have been identified in the colorful tissues of birds, ranging from common diet components like zeaxanthin, to novel methoxycarotenoids found only in feathers of the cotingas (LaFountain et al., 2015).

The diversity of carotenoids in coloration contrasts with the visual system, where the composition appears to be largely conserved among species. In the avian retina, specific types and amounts of carotenoids accumulate in the oil droplets of the different cone photoreceptors and provide spectral filtering that is predicted to facilitate color vision (Box 1). The cone oil droplets of at least 65 species have been examined to date, and 60 of these have been observed to have the full complement of oil droplet types (Box 1; Table S1). The spectral properties of the different droplet types are remarkably consistent among species; chromatographic analyses of avian retinas reveal that most birds have the same major carotenoids, including galloxanthin (an apocarotenoid; see Glossary), xanthophylls (i.e. lutein and zeaxanthin) and astaxanthin (a C4-ketocarotenoid; see Glossary) (Fig. 1; Box 1; Bhosale et al., 2007; Davies, 1986; Hart and Vorobyev, 2005; McGraw and Toomey, 2010; Stransky and Schulze, 1977; Toomey and McGraw, 2007; Toomey et al., 2015). Although there is general conservation of carotenoid types within the retina, there is evidence that the concentrations in the oil droplets vary among individuals (Ronald et al., 2017; Caves et al., 2020) and across the retina of an individual (Box 2; Table S1). Later, we will discuss how dietary and physiological processes, as well as the light environment (Box 2), influence carotenoid concentrations in the oil droplets and their subsequent visual functioning.

Shared mechanisms of carotenoid processing in avian color and vision

Once carotenoids are consumed, they must be taken up from the digestive tract and transported to the site of deposition. In this process, they may be metabolized into a diversity of forms. It seems a relatively small set of transporters, receptors and enzymes underlie the diversity of carotenoid-based coloration in birds (Funk and Taylor, 2019; Toews et al., 2017). The efficient uptake of carotenoids requires the apolipoprotein transporter scavenger receptor B1 (SCARB1). The disruption of SCARB1 function in canaries (*Serinus canaria*) results in the near-total absence of carotenoid pigmentation in the feathers and the retina (Toomey et al., 2017). Tracing studies indicate that all of the carotenoid types in the avian retina can be derived from two dietary precursors, lutein and zeaxanthin (Bhosale et al., 2007; Schiedt, 1998; Schiedt et al., 1985). Precise tracing of carotenoid precursors to ornamental pigments has not yet been performed; however, controlled carotenoid feeding experiments suggest that extensive metabolic transformations also underlie much of the diversity in integumentary carotenoids (McGraw, 2006; Morrison and Badyaev, 2016). Red coloration in many birds is produced through the accumulation of C4-ketocarotenoids (e.g. astaxanthin, 3-OH-echinenone); this same class of carotenoids pigments the R-type oil droplet of the long wavelength-sensitive (LWS) cone photoreceptor in the retina (Fig. 1). C4-Ketocarotenoids are not typically present in the diets of terrestrial birds but can be produced through the addition of a keto group at the 4 and/or 4′ positions of common yellow dietary carotenoids (Lopes et al., 2016; Schiedt, 1998). The enzyme cytochrome P450 2J19 (CYP2J19) has been identified as a key mediator of this transformation in both the plumage and retina (Fig. 2A–C; Lopes et al., 2016; Mundy et al., 2016).

CYP2J19-mediated C4-ketocarotenoid pigmentation of the cone oil droplets appears to be an ancient adaptation of the vertebrate visual system that was likely present in the common ancestor of birds and turtles (Twyman et al., 2016). This suggests a scenario where C4-ketocarotenoid metabolism initially evolved as a mechanism of spectral tuning in the visual system and was subsequently co-opted to produce

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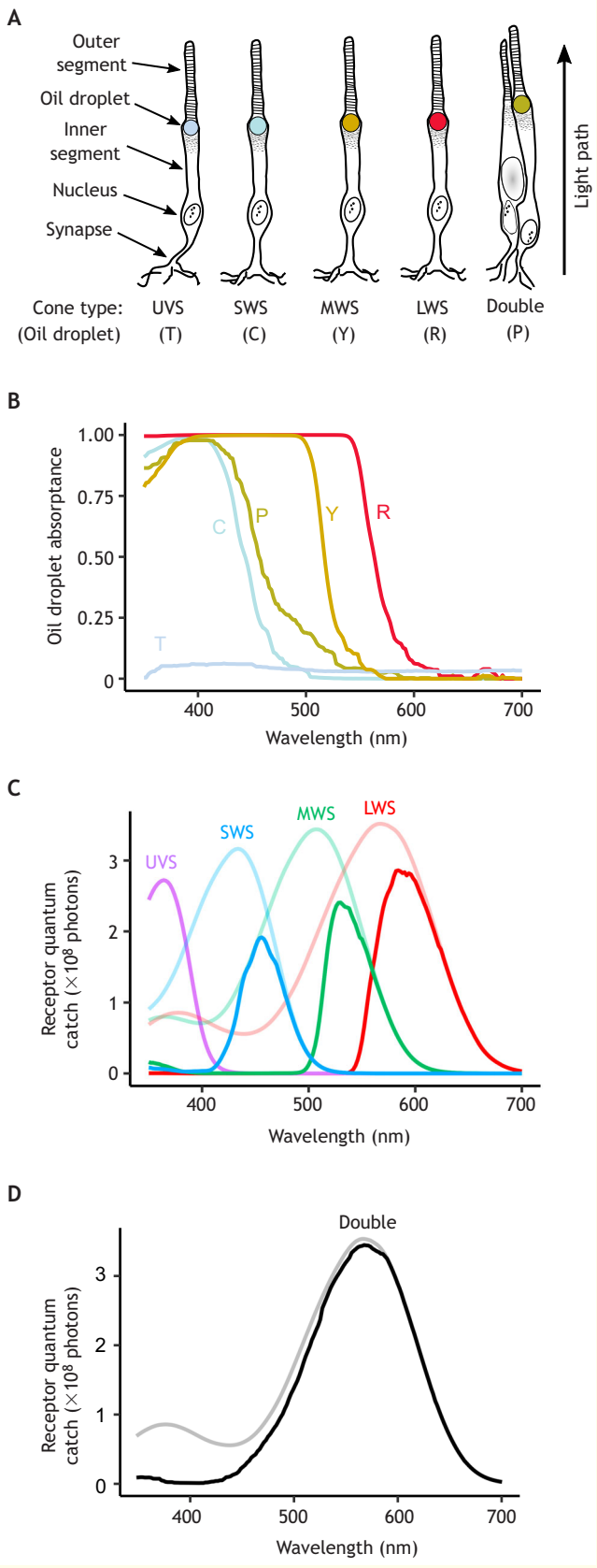

Box 1. Cone oil droplet form and function

Cone oil droplets are subcellular structures within the inner segment of the single cone photoreceptors and the principal member of the double cone (A), but are absent from the rod photoreceptors. The four avian single cone photoreceptors mediate color vision, double cones are thought to mediate achromatic discrimination, and rods mediate vision in dim light (Campenhausen and Kirschfeld, 1998; Hart and Hunt, 2007). The oil droplets function as refractive elements and spectral filters (as shown for the zebra finch in B) that modify the light reaching the photosensitive outer segments of the cones (Goldsmith et al., 1984; Stavenga and Wilts, 2014; Wilby and Roberts, 2017). Droplet filtering narrows the spectral bandwidth and reduces the sensitivity of the receptors. C and D show the predicted quantum catch (see Glossary), a measure of sensitivity, of the single and double cone photoreceptors with (saturated lines) or without (desaturated lines) the cone oil droplet filtering. The pigmented droplets function as long-pass cutoff filters that narrow the spectral sensitivity bandwidth of each cone subtype; this should enhance color discrimination and improve color constancy in changing light environments (Vorobyev, 2003; Vorobyev et al., 1998). However, filtering reduces receptor sensitivity by as much as 90% and may limit visual system performance in dim light (Gomez et al., 2014; Lind and Kelber, 2009a; Wilby and Roberts, 2017; Wilby et al., 2015).

Oil droplet filtering is matched to the sensitivity of the visual pigment of each cone subtype through the accumulation of specific types and concentrations of carotenoids (Arteni et al., 2019; Goldsmith et al., 1984; Toomey et al., 2015). For example, in the zebra finch, the ultraviolet-sensitive cones (UVS) contain an unpigmented oil droplet that is largely transparent (T-type), and the short wavelength-sensitive (SWS) and double cone contain a droplet (C-type and P-type, respectively) pigmented primarily with the apocarotenoid 3,4-dihydrogalloxanthin, which absorbs ultraviolet light. The medium wavelength-sensitive cone (MWS) contains a yellow (Y-type) oil droplet pigmented primarily with zeaxanthin, and the long wavelength-sensitive cone (LWS) contains a red (R-type) oil droplet pigmented primarily with astaxanthin (Goldsmith et al., 1984; Toomey et al., 2016). This distribution of carotenoid types is consistent among species, with the exception of the C-type droplet. The C-type droplet varies predictably with the spectral tuning of the UVS cone opsin (Hart and Vorobyev, 2005; Toomey et al., 2016). In species with a UV-shifted UVS cone opsin, 3,4-dihydrogalloxanthin is the major component of the C-type droplet, whereas in species with a violet-shifted UVS cone opsin, galloxanthin, with a relatively long wavelength-shifted absorbance spectrum, is the primary pigment (Toomey et al., 2016). These correlated changes are predicted to optimize color discrimination within the visual systems (Toomey et al., 2016). A–D adapted from Toomey et al. (2015).

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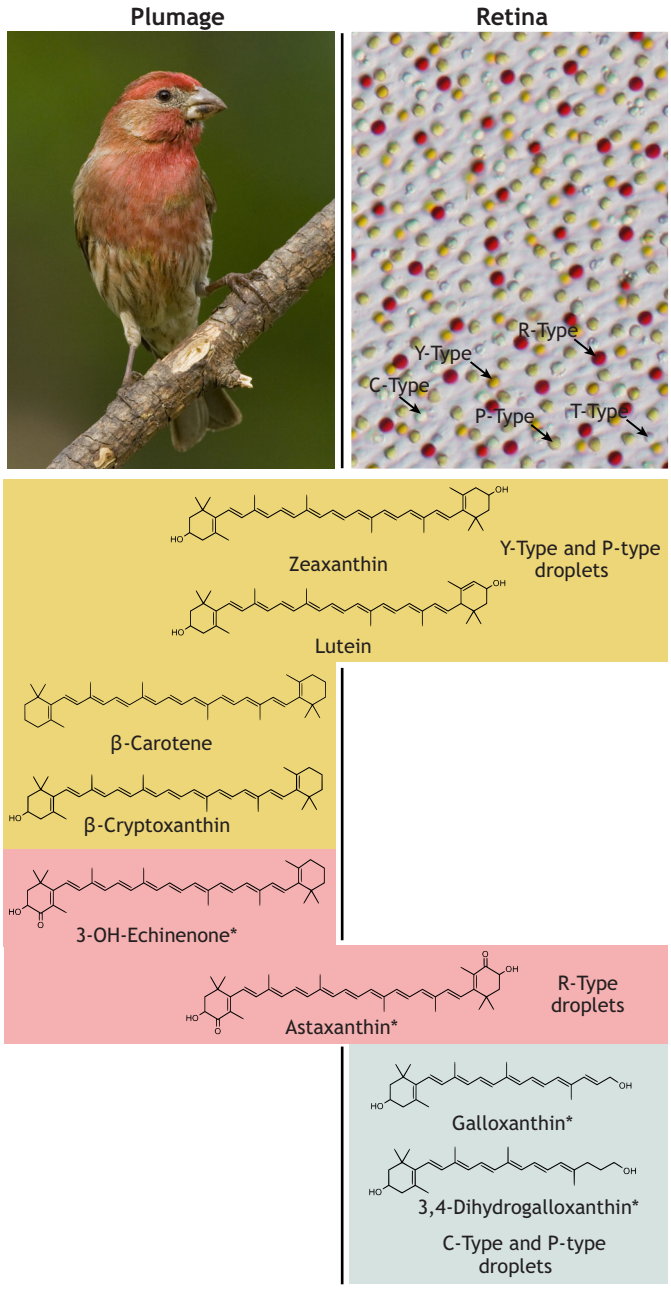

Fig. 1. The structures of major carotenoid types found in the house finch (*Haemorhous mexicanus*) plumage and retina. Shading color indicates the typical color the pigments impart and the shading extent indicates the pigment distribution across the plumage and retina. We have noted the cone oil droplet types where each carotenoid is a major component (Box 1). Note that T-type droplets do not contain carotenoids and do not have a spectral filtering function, but do have a role in the optics of the UVS cone. *Metabolically derived carotenoids; others are common components of the diet. Plumage photo courtesy of Geoff Hill. Retina image is a 600× micrograph from zebra finch (*Taeniopygia guttata*), a species with similar retinal carotenoid composition (Toomey et al., 2016).

red integumentary coloration (Lopes et al., 2016; Mundy et al., 2016; Twyman et al., 2016, 2018). Red oil droplets are present in nearly all bird species (Table S1); therefore, metabolism via CYP2J19 is likely occurring in the retinas of most species. This may explain the apparent evolutionary lability of red coloration: through the course of avian speciation and diversification, there are numerous transitions from yellow to red and back again (Friedman et al., 2014; Ligon et al., 2016; Prager and Andersson, 2010). The yellow to red to yellow transition might be a relatively simple matter of activating and inhibiting *CYP2J19* expression in the integument.

The conserved function of CYP2J19 in oil droplet pigmentation could constrain the diversification of carotenoid-based coloration. For example, selection on coloration might favor changes in CYP2J19 substrate specificity or expression that could also alter red cone oil droplet pigmentation in a way that compromises visual function, thus generating opposing selection pressures. These competing selective pressures may favor the duplication and subfunctionalization of CYP2J19, as has been observed in the zebra finch (*Taeniopygia guttata*) (Mundy et al., 2016). Zebra finches have two copies of *CYP2J19*, one of which is expressed in the integument (i.e. red beak) and one in the retina (Mundy et al., 2016). This subfunctionalization potentially relieves constraints imposed by visual function and might facilitate the diversification of carotenoid coloration. To date, this *CYP2J19* duplication has only been observed in zebra finches, but only a small fraction of species (n=43) have been examined (Emerling, 2018; Mundy et al., 2016; Twyman et al., 2018). As genomic resources become available, comparative analyses of *CYP2J19* copy number, expression and function will provide opportunities to investigate the role of constraint and subfunctionalization in bird color diversification.

Elaborate coloration is produced not only through the accumulation of carotenoids but also through their selective elimination. Recently, the enzyme β-carotene oxygenase 2 (BCO2) was identified as an important mediator of carotenoid degradation and tissue-specific, sexually dimorphic color patterning (Gazda et al., 2020a,b). BCO2 asymmetrically cleaves carotenoids, a first step in their degradation, but is also a key step in the production of the apocarotenoids that pigment the C- and P-type oil droplets of the avian visual system (Box 1; Fig. 2D,E; dela Seña et al., 2016; Lobo et al., 2012a; Toomey et al., 2016). The BCO2-mediated breakdown of carotenoids is also central to general carotenoid homeostasis. Knockout of *BCO2* in mice and zebrafish results in excessive carotenoid accumulation and oxidative damage to the mitochondria (Amengual et al., 2011; Guo et al., 2017; Lobo et al., 2012b; Wu et al., 2017, 2021). Thus, the evolution of BCO2 expression pattern and function is shaped by the balance of selective pressures on at least three distinct roles: (1) carotenoid homeostasis, (2) spectral tuning of the visual system and (3) coloration.

Are there shared constraints on carotenoid-based coloration and spectral filtering?

Diet alterations have profound effects on carotenoid-based coloration in birds (Blount and McGraw, 2008; Hill, 1992; McGraw, 2006; Svensson and Wong, 2011). Diet also affects the accumulation of carotenoids in the avian retina; however, the magnitude, time course and specificity of these effects are different from those seen for carotenoid-based plumage. Total dietary carotenoid deprivation can render the cone oil droplets colorless, but this manipulation must be carried out over multiple generations because maternally derived carotenoids are sufficient to produce long-lasting droplet pigmentation (Bowmaker et al., 1993; Meyer, 1971; Meyer et al., 1971; Wallman, 1979). In adult birds, carotenoid deprivation for at least 4 weeks is necessary to produce statistically significant declines in carotenoid concentration in the whole retina (Toomey and McGraw, 2010). Note that the relationship between whole-retina carotenoid concentration and the spectral filtering of cone oil droplets remains to be determined. In contrast, a single-day change in dietary carotenoid content during the molt can alter the coloration of carotenoid-pigmented plumage (Hill, 2002).

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Box 2. Light environment and carotenoid-based spectral filtering

Carotenoid-based spectral filtering imposes a trade-off between color discrimination and absolute sensitivity (Box 1); therefore, variation among species and individuals could reflect adaptive or plastic responses to the visual environment. The case for adaptive variation is clearest for owls and penguins, which have largely depigmented cone oil droplets along with a suite of other adaptations for dim-light vision (e.g. large eyes, increased density of rod photoreceptors, loss of some cone classes) (Alix et al., 2017; Bowmaker and Martin, 1978; Bowmaker and Martin, 1985; Gondo and Ando, 1995; Höglund et al., 2019; Wu et al., 2016). In fact, in some dim-light species, *CYP2J19* is a pseudogene, indicating that these species have entirely lost the capacity to produce the ketocarotenoids that pigment the R-type cone oil droplet (Emerling, 2018). Presumably, the loss of droplet pigmentation increases receptor absolute sensitivity, facilitating vision at night or deep underwater. Among diurnal terrestrial species, the relationship between droplet filtering and visual ecology is not yet clear. As more avian visual systems are characterized, comparative approaches may offer insights into the adaptive variation of spectral filtering. For example, we would predict that open-habitat species (e.g. marine or grassland) would have greater droplet spectral filtering compared with species inhabiting forest interiors.

There is evidence that the lighting environment can drive plastic responses in oil droplet spectral filtering within a species. Hart et al. (2006) observed that the pigment density of cone oil droplets is dependent on the light intensity chickens experience in their rearing environment. Birds reared in bright conditions develop cone oil droplets with significantly long wavelength-shifted spectra (Hart et al., 2006). Similar to diet manipulation studies, the most dramatic changes in spectral filtering are observed in the P-type oil droplet and the largest shifts are seen in the oil droplets of the ventral retina (Hart et al., 2006). Increased oil droplet pigment density in the ventral retina has been observed in a number of species (Table S1; Coyle et al., 2012; Knott et al., 2010). The increased pigmentation of ventral oil droplets may be an adaptation to the relatively higher levels of light impinging on this visual field, which is oriented skyward (Hart et al., 2006). Yet, the plasticity of oil droplet pigmentation in response to light environment may be limited to specific periods of development (see, for example, Toomey and McGraw, 2016). Taken together, the relationship between droplet spectral filtering and light exposure among species, individuals and even within the retina, suggests that subtle changes in droplet spectral filtering are functionally significant. Do animals in different environments (i.e. bright or dim lighting conditions) face differential trade-offs in the costs/benefits of oil droplet pigmentation? Do these shifts impact vision in general and the discrimination/perception of carotenoid-based colors in particular? If so, these light environment-driven shifts have the potential to contribute to classic sensory drive dynamics in the evolution of avian plumage coloration (Price, 2017).

In the retina, changes in dietary carotenoid content do not affect all retinal oil droplet types equally. Carotenoid supplementation in zebra finches and crimson rosellas (*Platycercus elegans*) primarily increases pigmentation in the double cone P-type droplets rather than all oil droplet types (Knott et al., 2010). Similarly, carotenoid supplementation in house finches primarily increases the concentration of apocarotenoids, the major pigments of the P-type droplet (Toomey and McGraw, 2010, 2011; Toomey et al., 2015). A more recent diet deprivation study in zebra finches found no changes in R-type oil droplet pigmentation (Caves et al., 2020). Together, these studies suggest that carotenoid-based spectral filtering may be buffered against short-term changes in diet. Moreover, when changes do occur, the impact might be greatest on achromatic discrimination mediated through the double cones with their P-type oil droplet.

Although laboratory manipulations of dietary carotenoids can lead to significant changes in coloration and oil droplet spectral filtering, it is questionable whether carotenoid availability varies to this degree in the natural environment (Hudon, 1994; Koch and Hill, 2018). Here, it is informative to consider the relative amounts of carotenoid involved in pigmenting the feather versus the cone oil droplets. Koch and Hill (2018) estimate that 41 µg of carotenoids are required to pigment the plumage of a male house finch. We estimate that a house finch requires only 2.33 µg of carotenoids to completely pigment the cone oil droplets in their retinas (Toomey and McGraw, 2009). Thus, even if carotenoids are environmentally limited, the requirements for the development and maintenance of oil droplet spectral filtering might be more easily met than the requirements for plumage coloration.

Nevertheless, even if the diet is sufficient, carotenoid accumulation in the cone oil droplets might be constrained by allocation to other physiological functions. Carotenoid pigments have been implicated as antioxidants and immunomodulators that promote the physiological condition of an individual (Lozano, 1994; Møller et al., 2000; Pérez-Rodríguez, 2009; von Schantz et al., 1999). Therefore, the pool of carotenoids acquired from the diet might be subject to trade-offs among physiological functions. These trade-offs are often invoked to explain the information content, honesty and evolution of carotenoid-based colors, but their significance is a matter of current debate (reviewed in Koch and Hill, 2018). In house finches, retinal carotenoid levels positively correlate with body mass normalized to body size, suggesting that carotenoid accumulation in the visual system is somehow linked to physiological condition (Toomey and McGraw, 2009). In captive finches, repeated immune system activation with large doses of phytohemagglutinin and lipopolysaccharide (LPS) results in the depletion of carotenoids in the retina (Toomey et al., 2010). LPS not only induces immune activation but also increases oxidative damage in birds (Armour et al., 2020). Thus, these results may reflect the influence of both immunological and antioxidant processes. Interestingly, as with diet, immune system activation primarily affects apocarotenoid levels in captive house finches (Toomey et al., 2010). Yet, it is not clear how well these laboratory results generalize to infections in natural populations. Wild house finches infected with *Mycoplasma gallisepticum*, which causes inflammation of the eye conjunctiva, show no significant differences in retina carotenoid accumulation compared with healthy birds of the same population (McGraw et al., 2013).

Geoff Hill and colleagues have proposed that the condition dependence of carotenoid-based colors derives from a linkage between vital cellular processes and the metabolic transformations of carotenoids, such as C4-ketocarotenoids (Hill, 2014; Hill et al., 2019). In support of this hypothesis, the C4-ketocarotenoid plumage color expression in house finches is correlated with mitochondrial efficiency (Hill et al., 2019); additionally, manipulations of mitochondrial function have been shown to impact C4-ketocarotenoid coloration of other song bird species (Cantarero and Alonso-Alvarez, 2017; Cantarero et al., 2020). Whether and how mitochondrial function is linked to carotenoid metabolism and accumulation in the avian retina is not known and represents an exciting avenue of future research.

Current evidence suggests that diet and physiological stressors may have subtle effects on carotenoid-based spectral filtering in the visual system. To link vision and coloration through a common condition dependence of carotenoids requires that such subtle effects have substantial impacts on color vision. To explore this possibility, we first review the current evidence for carotenoid-mediated changes in vision, then use models of avian vision to examine whether and how retinal carotenoid levels might impact the perception of carotenoid-based plumage.

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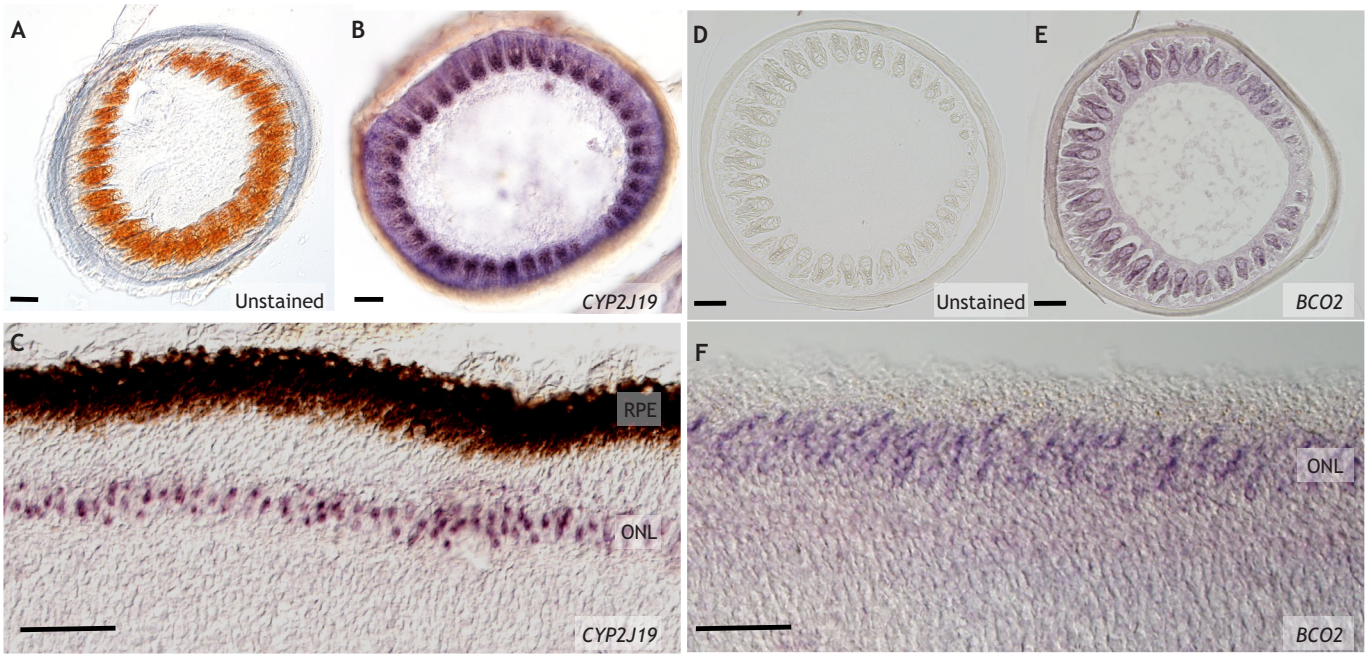

Fig. 2. CYP2J19 and BCO2 mediate carotenoid metabolism in the colorful plumage and retinas of birds. (A) Unstained section through the regenerating feather follicles of a red factor canary, a breed with red C4-ketocarotenoid-based plumage coloration that was produced by crossing the common canary (*Serinus canaria*) with the red siskin (*Spinus cucullata*). The red–orange color in the developing feather barb ridges comes from the accumulated C4-ketocarotenoid pigments. (B) *In situ* hybridization in the same feather follicle shows *CYP2J19* (purple) is expressed in the ketocarotenoid-accumulating feather tissue. (C) *In situ* hybridization in a cross-section of the developing chicken retina shows that *CYP2J19* (purple) localizes to a subset of photoreceptor cells, a pattern consistent with the selective accumulation of ketocarotenoids in the long wavelength-sensitive (LWS) cones. RPE, retinal pigment epithelium; ONL, outer nuclear layer that contains photoreceptor nuclei. (D) Unstained section through the regenerating feather follicles of a female mosaic canary, a breed with sexually dimorphic carotenoid-based plumage coloration. Carotenoid pigments are absent in most of the female feathers, hence the white/translucent appearance. (E) *In situ* hybridization in the same feather follicle shows *BCO2* (purple) is expressed in the developing feather, a pattern consistent with a role in the degradation and elimination of carotenoids. (F) *In situ* hybridization for *BCO2* in the developing chicken retina shows expression in a subset of photoreceptors that is consistent with its proposed role in apocarotenoid pigmentation in short wavelength-sensitive 2 (SWS2) and double cones. Note the RPE was removed from this preparation of the retina prior to staining. Scale bars, 50 µm. Figures are adapted from Lopes et al. (2016); Toomey et al. (2016); Gazda et al. (2020b).

Carotenoid-based spectral filtering, vision and behavior

A handful of studies have investigated the impacts of carotenoid supplementation on visually mediated behaviors and color discrimination in birds. Toomey and McGraw (2011) altered retinal carotenoid levels in adult house finches through diet supplementation, assessed visually mediated foraging behavior by presenting colorful food items in a matrix of achromatic distracters, and manipulated contrast through changes in lighting. Surprisingly, they found that increased accumulation of carotenoids in the retina – specifically, 3,4-dihydrogalloxanthin and galloxanthin – led to a decline in visual foraging performance. In this experiment, both chromatic and achromatic cues were available to the finches and the increased spectral filtering of the double cone may have reduced sensitivity to achromatic cues (Toomey and McGraw, 2011). In a more precisely controlled experiment, Lim and Pike (2016) trained young quail to discriminate paper cones printed with yellow to orange patterns. Carotenoid supplementation significantly improved discrimination of contrasting colors close to the thresholds of discrimination predicted by the receptor noise-limited model (see Glossary) of avian color discrimination. This result suggests that increased carotenoid-based spectral filtering enhances color discrimination. However, neither Lim and Pike (2016) nor Toomey and McGraw (2011) directly measured droplet spectral filtering, and it is possible that other physiological effects of carotenoids could be contributing to these changes in behavior. Recently, Caves et al. (2020) provided direct evidence of a potential link between droplet spectral filtering and color vision in zebra finches. They focused on colors resembling the sexually selected carotenoid-based beak coloration of the zebra finch, and found discrimination across a previously defined categorical boundary was positively correlated with individual variation in R-type droplet filtering (Caves et al., 2020). This intriguing result suggests that carotenoid-based spectral filtering interacts with higher-order processes of color perception in ways that we do not yet fully understand.

The only study to directly investigate carotenoids in the visual system and mate choice for carotenoid-based coloration also lacks direct measures of oil droplet filtering. In aviary experiments, Toomey and McGraw (2012) supplemented the diets of female house finches to increase carotenoid levels in the retina, and assessed their preference for the carotenoid-based plumage in males manipulated to simulate a range from drab yellow to deep red. As expected, females preferred the reddest males, but preference and discrimination were not significantly influenced by carotenoid supplementation or correlated with carotenoid levels in the whole retina (Toomey and McGraw, 2012). This suggests that diet-driven variation might not influence discrimination of carotenoid-based signals; however, the link between whole-retina carotenoid measures and spectral filtering at the photoreceptor level has not yet been established. This link could be confounded by regional variation in droplet pigmentation across the retina and/or carotenoid accumulation outside the oil droplets, although the latter is unlikely. Clearly, refined experimental approaches are needed to address these questions. One way forward is to precisely specify experimental predictions based on current models of avian visual physiology. The modeling exercise we present in the next section (see also Appendix) is an effort to explore how changes in oil

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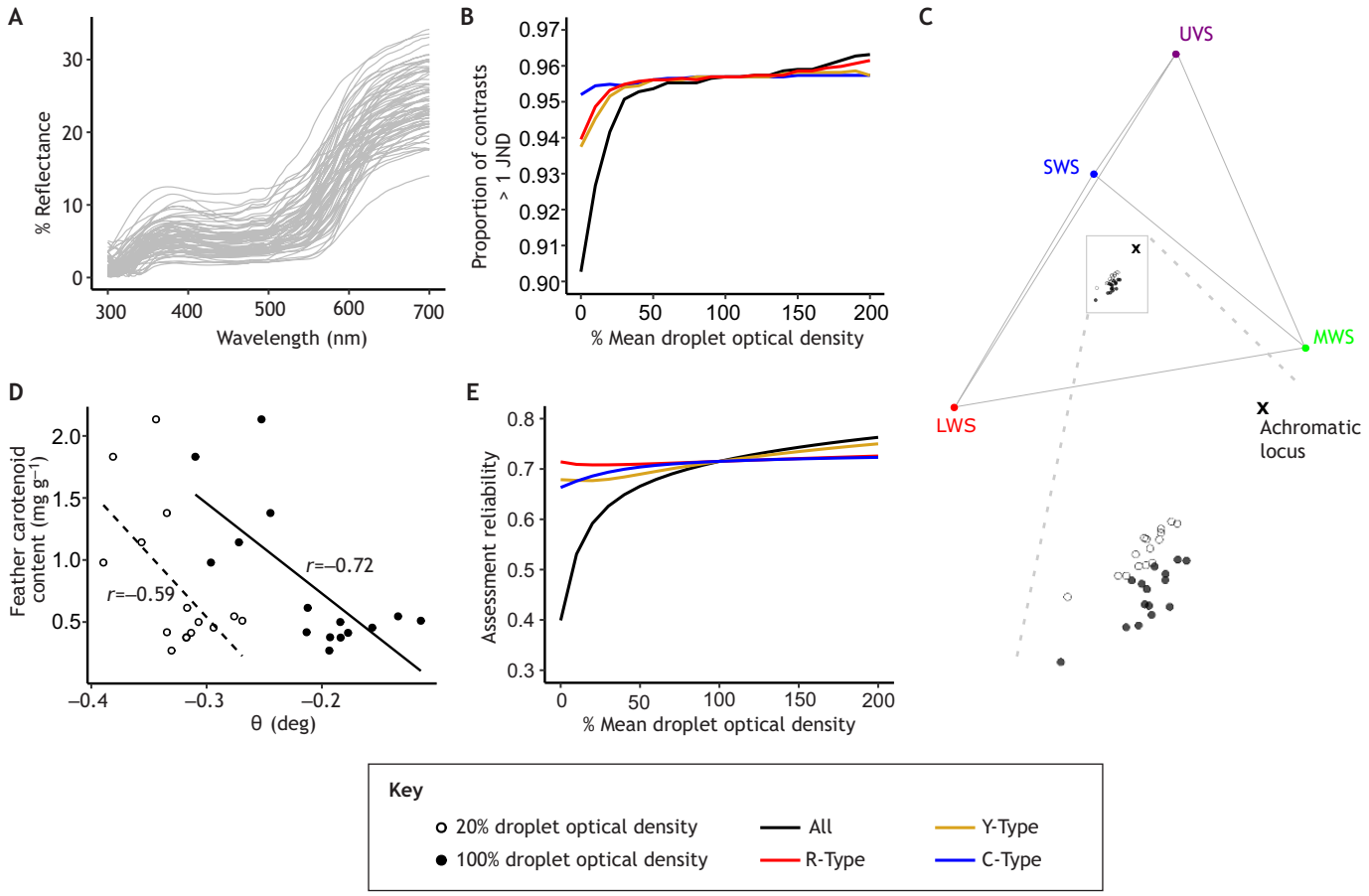

Fig. 3. Changing the carotenoid-based spectral filtering of the cone oil droplets has little impact on chromatic contrast among carotenoid-based plumage spectra, but alters the relationship between color space location and feather carotenoid content. (A) The reflectance spectra of carotenoid-pigmented breast plumage of 70 male house finches. (B) The proportion of pairwise contrasts among the plumage spectra in A that have a chromatic contrast of >1 JND for a visual system modeled with varying optical densities of the three pigmented single cone oil droplets together or individually. We varied optical density of the oil droplets from 0 to 200% of the measured mean. (C) The predicted tetrahedral color space location of selected plumage spectrum (n=15) for a visual system with oil droplets of the mean measured optical density (filled circles) or 20% of the mean (open circles). The achromatic center of color space is denoted with a cross. It is from this point that angle θ is measured in the LWS–MWS–SWS plane. (D) The relationship between tetrahedral color space location, θ and feather carotenoid content of selected plumage spectra for a visual system modeled with oil droplets with the measured mean optical density or 20% of the measured density. (E) The reliability of feather carotenoid content assessment measured as the absolute value of Pearson correlation between tetrahedral color space location, θ and direct measures of feather carotenoid content, for a subset of spectra (n=15), viewed through a visual system with oil droplet density varied as in B.

droplet filtering might affect the discrimination and assessment of carotenoid-based colors.

Predicting the impact of carotenoid-based spectral filtering

Visual models that take into account avian visual physiology (Maia et al., 2013; Maia et al., 2019; Stoddard and Prum, 2008; Vorobyev and Osorio, 1998; Vorobyev et al., 1998) offer an opportunity to make precise predictions about how carotenoid-based spectral filtering impacts vision. These models provide perceptual estimates of the location of visual stimuli in color space (see Glossary) and the chromatic and achromatic contrasts (see Glossary) among stimuli (Endler and Mielke, 2005; Goldsmith, 1990; Stoddard and Prum, 2008). The distance in color space can be measured in values of just-noticeable difference (JND), where 1 JND is equated to the threshold of discrimination set by receptor noise (Vorobyev and Osorio, 1998; Vorobyev et al., 1998; but see Cheney et al., 2019; Hempel de Ibarra et al., 2002, for deviations from model). These models also take into account the ambient light in the environment and parameters used to describe the visual system. Most often these parameters include: (1) the sensitivity of the visual pigments, (2) the spectral filtering of the oil droplets in the SWS, medium wavelength-sensitive (MWS) and LWS cones, and (3) the density of photoreceptors.

Ronald et al. (2017) used this approach to explore how individual differences in both oil droplet absorbance and photoreceptor density among brown-headed cowbird females might impact color discrimination. These differences in visual physiology resulted in statistically significant, but relatively small, changes in predicted chromatic contrast of male plumage coloration against vegetative and plumage backgrounds (Ronald et al., 2017). The mean variation in the predicted discrimination of females was about 14% (from 10.00±0.06 to 11.50±0.07 JND; Ronald et al., 2017). Interestingly, the mean variation in predicted achromatic contrast was around 40% (from 4.36±0.09 to 10.49±0.21 JND), suggesting that achromatic contrast discrimination may be more sensitive to changes in these visual parameters (Ronald et al., 2017). Despite the relatively small changes in chromatic contrast values found in this study, behavioral evidence from *Anolis sagrei* lizards suggests that relatively small differences in chromatic contrast (e.g. 2–4 JND) can alter the mean probability of detection (Fleishman et al., 2016).

Several independent modeling exercises have systematically varied the cone oil droplet spectral filtering component to explore its impact on predicted color discrimination (Bitton et al., 2017; Lind and Kelber, 2009b; Lind et al., 2017). Lind and Kelber (2009b) and Lind et al. (2017) examined how these shifts affected the predicted contrasts among a small set of natural spectra (e.g. seeds, leaves) or simulated short, medium and long wavelength-enriched spectra, respectively. They predicted limited changes in color discrimination when oil droplet filtering is shifted and other parameters are held constant (Lind and Kelber, 2009b; Lind et al., 2017). Bitton et al. (2017) varied oil droplet filtering and other components of the model and calculated chromatic contrasts between the plumage spectra of males and females of 70 galliform birds. Although shifts in oil droplet filtering usually had small effects, for certain comparisons the effects were large (i.e. >7 JND; Bitton et al., 2017). Thus, there may be specific contexts where shifts in droplet filtering have a large impact on visual function. Might the discrimination of carotenoid-based colors be one such context?

To address this possibility, we adapted the modeling approaches of Lind et al. (2017) to ask how changes in oil droplet spectral filtering impact the discrimination of the carotenoid-based plumage coloration of male house finches. To do this, we used male house finch breast plumage reflectance spectra (n=70) from previous studies (Butler et al., 2011; Toomey and McGraw, 2009), house finch cone oil droplet absorbance spectrum measurements, and visual pigment spectral sensitivities of a related passerine species (canary; Das et al., 1999). We modeled cone oil droplet transmission based on the expanded droplet absorbance spectrum as a function of varying optical density (see Glossary) from 0 to 200% of the observed mean (see Appendix for methods). This generated a range of droplet spectral filtering that overlapped and extended beyond the variation reported among bird species (Table S3). For each configuration of droplet filtering, we calculated all pairwise chromatic contrasts among the 70 individuals, 2415 contrasts in all, and examined the proportion of these contrasts that exceeded a discrimination threshold of 1 JND (Fig. 3A,B). We found that the large majority of contrasts exceed threshold regardless of the level of oil droplet filtering (Fig. 3B). At very low oil droplet optical densities (<30% of the mean), there was a decline in the proportion of contrasts exceeding threshold, especially when the densities of all three droplet classes were altered simultaneously. Relative to typical droplet filtering, complete removal of droplet filtering increased the number of contrasts below threshold by 2.3-fold. This is consistent with Vorobyev et al. (1998), who calculated that removing droplet filtering causes a 1.4- to 2.4-fold increase in indiscriminable contrasts among a wider diversity of plumage spectra. Nevertheless, it remains to be determined whether and how often such carotenoid depletion occurs among wild birds. Changes in the spectral filtering of the P-type oil droplet had little impact on the achromatic contrasts among our sample of plumage spectra (Fig. S1). Overall, the majority of contrasts among colorful males are predicted to remain discriminable despite changes in oil droplet spectral filtering. However, our model assumes conditions of bright sunlight and that the photoreceptors are adapted to a uniform white background. We encourage the exploration of the full parameter space of the model; this may reveal specific conditions, such as dim light, where droplet filtering could have greater impacts on discrimination (see Box 2).

Color vision involves not only the discrimination of contrast but also the acquisition of information about the properties of objects and surfaces. In house finches, females select mates on the basis of carotenoid pigment plumage coloration, which is hypothesized to reveal information about the quality of a potential mate (Hill, 2002). The color of carotenoid pigment plumage is largely determined by the concentration of pigment in the feather (Butler et al., 2011; Inouye et al., 2001; Saks et al., 2003; Shawkey et al., 2006). Therefore, we wondered whether and how changes in the spectral filtering of the cone oil droplets might impact the assessment of feather carotenoid content during mate choice.

To explore this question, we used the modeling approach described above and calculated the tetrahedral color space location of a sample of house finch spectra (n=15) from feathers whose carotenoid content had been directly measured with HPLC (Butler et al., 2011). We then examined how well color space location predicted feather carotenoid content. We focused our analysis on the θ component, which is the angle of the vector extending from the achromatic center of color space to the point defined by a given plumage spectrum's relative stimulation of the SWS, MWS and LWS cone photoreceptors (Stoddard and Prum, 2008). A previous analysis of this dataset with mean values of oil droplet filtering showed that θ is significantly negatively correlated with plumage carotenoid content (Butler et al., 2011). Furthermore, this variable has also been shown to be a better predictor of the carotenoid content in feathers than measures of chromatic contrast (Butler et al., 2011).

We found that changes in oil droplet filtering shifted the color space location of the plumage spectra and altered the linear relationship between color space angle θ and feather carotenoid content (Fig. 3C–E). Reducing the optical density of all three cone oil droplets below the measured mean substantially weakened the correlation between θ and feather carotenoid content from a Pearson's correlation of r=0.71 at mean droplet density to r=0.40 when droplet filtering was entirely removed. In contrast, doubling the droplet density above the mean produced a small improvement (r=0.76) and changes in the filtering of single droplet classes appeared to have little impact on the relationship between θ and feather carotenoid content (Fig. 3E). Isolated changes to a single droplet class shifted all of the spectra along similar linear trajectories through color space and largely maintained their relative positions. In contrast, changing multiple droplets resulted in non-linear shifts and reordering of the relative positions of the spectra (Fig. S2). Thus, the general depletion of carotenoids from all of the cone oil droplets may impact the reliability of color as an indicator of feather carotenoid content. Individuals with sub-optimal droplet pigmentation levels may be limited in their capacity to assess carotenoid-based signals, leading to greater uncertainty and more errors during signal evaluation. These results are intriguing as they could provide a physiological basis for some of the variation in mating preferences in birds that use carotenoid-based signals.

The predicted changes we found in the reliability of carotenoid assessment may help explain the observations of individual variation in the strength of categorical discrimination across a carotenoid-like color continuum. Caves et al. (2020) found that (1) there is individual variation in red cone oil droplet absorbance in female zebra finches and (2) birds with relatively high levels of droplet pigmentation are better able to discriminate across a categorical boundary in color space. Our results suggest that changes in droplet filtering shift the location of spectra in perceptual color space. If these shifts move spectra from one side to another of a categorical boundary they could facilitate or confound categorical discriminations depending on the nature of the change. These recent results (Caves et al., 2020), combined with earlier work from Ronald et al. (2017), suggest that there is inherent variation in the oil droplet absorbance properties in both captive and wild songbird species. Furthermore, these individual differences can produce both predicted changes in measures of chromatic contrast discrimination (as demonstrated here and by Ronald et al., 2017) and behavioral tasks linked to mate-

choice decisions (Caves et al., 2020). Thus, the physiological and environmental constraints on carotenoid-based spectral filtering in the avian visual system discussed above might create individual variation around categorical boundaries. Such variation has been suggested to be a feature that can resolve the paradoxical evolution of continuously varying signaling traits, such as carotenoid-based colors, in response to selection by receivers applying categorical discrimination thresholds to those traits (Peniston et al., 2020).

Conclusions and future prospects

The role of carotenoids in both the coloration and color vision of birds has led one of us (M.B.T.) to speculate that '...plumage colouration and colour perception may be linked through a common biochemical mechanism' (Toomey and McGraw, 2009). Now, more than a decade later, we have evidence that the same enzymes and transporters mediate the expression of both carotenoid-based coloration and visual spectral filtering. The conservation of carotenoid-based spectral filtering among birds suggests that some of these mechanisms first evolved in the visual system and have subsequently been co-opted for color expression (Lopes et al., 2016; Twyman et al., 2016). The next step in understanding this linkage will be to determine the gene regulatory changes that underlie this co-option. This will allow us to dissect the mechanics of how diversifying selection pressures on plumage color are balanced with stabilizing selection on the visual system.

Carotenoid-based colors of birds are classic examples of condition-dependent traits (McGraw and Blount, 2009), but it seems that carotenoid-based spectral filtering in the visual system does not share the same degree of condition dependence. The effects of diet and health on carotenoid accumulation in the retina are subtle and specific. Also, oil droplet pigmentation is distinctly sensitive to the light environment, which could link color and vision through classic sensory drive processes (Box 2; Price, 2017). Our modeling exercise suggests that changes in cone oil droplet filtering have little impact on the discrimination among carotenoid-based colors, but might alter how these signals are assessed. Thus, both the expression and reliable assessment of carotenoid-based color may be shaped by the availability and physiology of carotenoids in ways that might drive the elaboration and diversity of these signals. However, our models are relatively limited in scope and encompass a narrow set of conditions, and the effects we observed are generally subtle. Whether such effects are realized in nature is an open question, and much work is required to determine their biological significance. What is needed now is a better understanding of how droplet filtering varies among individuals and the functional implications of this variation through behavioral studies of color discrimination and assessment. These efforts will clarify whether and how the unique biology of carotenoids contributes to trait–preference co-evolution between senders and receivers.

Appendix

Vision modeling methods

To examine the impact of changes in the carotenoid-based spectral filtering of the cone oil droplets on discrimination of carotenoid-based plumage spectra, we adapted the approaches of Lind et al. (2017) and Butler et al. (2011).

Approach 1 – color discrimination with varying droplet optical density

Cone oil droplet filtering spectra

To model the transmittance of the cone oil droplets, we used the absorbance spectra of expanded house finch oil droplets (Dryad digital repository: doi:10.5061/dryad.pg4f4qrnr) and calculated transmittance following Goldsmith and Butler (2003):

$$P(\lambda) = 10^{-aD(\lambda)}, \quad \text{(A1)}$$

where $D(\lambda)$ is the normalized absorbance spectrum and a is optical density. The mean estimated optical density of the droplets was C-type 1.9, Y-type 3.5, R-type 11.7, and P-type 2.1. To vary oil droplet density, we multiplied this mean value by a range of values from 0 to 2 at an interval of 0.1. Note that the spectrum measurements below 350 nm contained large amounts of noise as a result of limitations of the illuminant and optics of the microspectrophotometry system. For this exercise, we chose to fix the absorbance from 300 to 350 nm to the measured value at 350 nm.

Receptor quantum catch

We calculated the quantum catch (N) of each cone photoreceptor class (i) following Lind et al. (2017):

$$N_i(\lambda) = \Delta t(\frac{\pi}{4})^2 R^2 d^2 K O(\lambda) P(\lambda)(1 - e^{-kA(\lambda)l}) L(\lambda), \quad \text{(A2)}$$

where R is the acceptance angle, d is pupil diameter, K is the quantum transduction efficiency, O is ocular media transmittance, P is the spectral transmittance of oil droplets, k is the absorption coefficient, A is the normalized absorbance of the visual pigment, l is the length of the cone outer segment and L is the plumage reflectance spectrum multiplied by the illuminant spectrum.

Chromatic contrast

We calculated the receptor-specific contrast between two stimuli:

$$\Delta f_i = \ln\left(\frac{N_{i,L1}}{N_{i,L2}}\right). \quad \text{(A3)}$$

We calculated noise for each receptor class including Weber noise and photon shot noise assuming that intrinsic noise v_i=0.1:

$$e_i = \frac{\sqrt{\left(\frac{v_i}{\sqrt{n_i}}\right) N_i^2 + N_i}}{N_i}. \quad \text{(A4)}$$

Here, n is the abundance of the given receptor given in Table S2, and N is the mean quantum catch for spectra $L1$ and $L2$. We fixed the summed noise of all four cone classes to a combined value of 0.4.

The subscripts indicate cone type: 1, UVS cones; 2, SWS cones; 3, MWS cones; and 4, LWS cones.

We calculated chromatic contrast for all pairwise contrasts of the carotenoid-pigmented breast plumage of 70 male house finches. Our analyses were run in R 4.0 (http://www.R-project.org/) with tools from 'tidyverse' (Wickham et al., 2019). Code, model parameters, plumage and droplet spectra data are available in Table S2 and from the Dryad digital repository (doi:10.5061/dryad.pg4f4qrnr).

Finally, we calculated chromatic contrast:

$$\Delta S^2 = \frac{((e_1e_2)^2(\Delta f_4 - \Delta f_3)^2 + (e_1e_3)^2(\Delta f_4 - \Delta f_2)^2 + (e_1e_4)^2(\Delta f_3 - \Delta f_2)^2 + (e_2e_3)^2(\Delta f_4 - \Delta f_1)^2 + (e_2e_4)^2(\Delta f_3 - \Delta f_1)^2 + (e_3e_4)^2(\Delta f_2 - \Delta f_1)^2)}{((e_1e_2e_3)^2 + (e_1e_2e_4)^2 + (e_1e_3e_4)^2 + (e_2e_3e_4)^2)}. \quad \text{(A5)}$$

Achromatic contrast

We assumed that achromatic contrast discrimination is mediated by the double cone photoreceptor (Campenhausen and Kirschfeld, 1998; Osorio et al., 1999) and modified the cone catch, noise and contrast calculations described above for a single receptor with an LWS opsin and filtered by the P-type droplet.

Approach 2 – color space location and feather carotenoid content

To examine the impact of cone oil droplet spectral filtering on the relationship between the predicted color space location and the carotenoid content of colorful plumage, we reanalyzed house finch plumage data from Butler et al. (2011). We varied cone oil droplet spectral filtering and calculated cone spectra sensitivity as described above. Following Stoddard and Prum (2008) we normalized cone sensitivity to an integral of one. For each configuration of oil droplet filtering, we calculated the relative quantum catch of each of the four cone classes for each of the plumage spectra and calculated its tetrahedral color space location in Cartesian coordinates as follows:

$$x = \frac{1 - 2s - m - u}{2}\sqrt{\frac{3}{2}}, \quad \text{(A6)}$$

$$y = \frac{-1 + 3m + u}{2\sqrt{2}}, \quad \text{(A7)}$$

$$z = u - 1/4. \quad \text{(A8)}$$

We then calculated the angle θ from the achromatic center of color space to the point in color space predicted for each plumage spectrum:

$$\theta = \arctan\left(\frac{y}{x}\right). \quad \text{(A9)}$$

Finally, we calculated the Pearson correlation coefficient between the calculated θ and direct measures of feather carotenoid content for each configuration of oil droplet filtering.

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Competing interests

The authors declare no competing or financial interests.

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Data availability

Data are available from the Dryad digital repository (Toomey and Ronald, 2021): dryad.pg4f4qrnr

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