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SYMPOSIUM ARTICLE

Mechanisms of Carotenoid Metabolism: Understanding the Links between Red Coloration, Cellular Respiration, and Individual Quality

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Synopsis In many species of birds, red carotenoid coloration serves as an honest signal of individual quality, but the mechanisms that link carotenoid coloration to animal performance remain poorly understood. Most birds that display red carotenoid coloration of feathers, bills, or legs ingest yellow carotenoids and metabolically convert the yellow pigments to red. Here, we review two lines of investigation that have rapidly advanced understanding of the production of red carotenoid coloration in birds, potentially providing an explanation for how red coloration serves as a signal of quality: the identification of the genes that enable birds to be red and the confirmation of links between production of red pigments and core cellular function. CYP2J19 and BDH1L were identified as key enzymes that catalyze the conversion of yellow carotenoids to red carotenoids both in the retinas of birds for enhanced color vision and in the feathers and bills of birds for ornamentation. This CYP2J19 and BDH1L pathway was shown to be the mechanism for production of red coloration in diverse species of birds and turtles. In other studies, it was shown that male House Finches (*Haemorhous mexicanus*) have high concentrations of red carotenoids within liver mitochondria and that redness is positively associated with mitochondrial function. These observations suggested that the CYP2J19 and BDH1L pathway might be tightly associated with mitochondrial function. However, it was subsequently discovered that male House Finches do not use the CYP2J19 and BDH1L pathway to produce red pigments and that both CYP2J19 and BDH1L localize in the endoplasmic reticulum, not the mitochondria. Thus, we have the most detailed understanding of links between cellular function and redness in a bird species for which the enzymes to convert yellow to red pigments remain unknown, while we have the best understanding of the enzymatic pathways to red in species for which links to cellular function are largely unstudied. Deducing whether and how signals of quality arise from these distinct mechanisms of ornamental coloration is a current challenge for scientists interested in the evolution of honest signaling.

Among the array of ornamental traits displayed by animals, red coloration produced by carotenoid pigments has been a particular focus of study by scientists interested in signaling in the context of mate choice (Hill 2002a; Blount and McGraw 2008; Svensson and Wong 2011). In many fish (Houde 1997), birds (Hill 2006a), and lizards (Kwiatkowski et al. 2002; Sullivan and Kwiatkowski 2007), research has shown that females are attracted to males with more saturated and red-shifted carotenoid displays. Moreover, in studies of diverse vertebrate taxa, redder individuals perform better than males with drabber coloration (Hill 2006b; Simonset al. 2012; Weaver et al. 2018). More specifically, redness predicts immunocompetence with redder individuals both resisting pathogens more effectively (Folstad et al. 1994; Brawner et al. 2000; Hill et al. 2004) and recovering from infection faster (Hill and Farmer 2005). Infection by a pathogen, or experimental

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immune stimulation, also decreases expression of red carotenoid coloration (Thompson et al. 1997; Faivre et al. 2003; Hill et al. 2004; Johansen et al. 2019). Redder individuals survive at a higher rate (Hill 1991; Pike et al. 2007; Simonset al. 2012; Simons et al. 2016; Cantarero et al. 2019; Fernández-Eslava et al. 2022; Romero-Haro et al. 2024), and redder males show lower levels of stress hormone (Duckworth et al. 2001; Alonso-Alvarez et al. 2008; Fitze et al. 2009) and less oxidative damage (Alonso-Alvarez and Galvan 2011; Simonset al. 2012) than less red males. Red carotenoid-based coloration has therefore emerged as an example of an honest signal of individual quality used in courtship communication.

The question that has perplexed generations of evolutionary biologists is: how can such honest signaling work (Hamilton and Zuk 1982; Smith 1991)? The intense pressures of sexual selection should favor the decoupling of quality and ornamentation and drive all males to uniformly exaggerated red coloration, yet red color expression remains variable within populations and across the life of individuals (Biernaskie et al. 2014; Higham 2014; Weaver et al. 2017). Discoveries related to the biochemical and cellular processes that underlie the production and display of red carotenoid pigments are generating new testable hypotheses regarding the fundamental nature of honest signaling via red carotenoid pigments (Toews et al. 2017; Weaver et al. 2017). Many questions related to color signaling remain to be answered, and at present, investigations into the mechanisms of carotenoid signaling are turning up more unexpected outcomes than clear answers (Hill 2022; Lindsay et al. 2022). Nevertheless, a path toward a new understanding of how red carotenoid coloration provides an honest signal of individual quality is taking shape. In this review, we synthesize the latest discoveries related to honest signaling via red carotenoid coloration and outline the key data that are needed to push investigations forward.

The CYP2J19/BDH1L pathway

With very few exceptions, animals cannot synthesize carotenoid pigments *de novo*; carotenoid pigments used in color displays must be ingested (Maoka 2020). Thus, acquisition of carotenoids in diet is a key component of the production of carotenoid-based color displays (Hill et al. 1994; McGraw et al. 2001). The most common and abundant carotenoids that are available in the diets of most terrestrial animals are the same carotenoids that are part of a typical human diet: β-carotene, lutein, zeaxanthin, and β-cryptoxanthin (Hill and Johnson 2012a; Lafountain et al. 2015). These carotenoid types are essential components of the photosystems of green plants (Sandmann 2021) and therefore readily available in any ecosystem with abundant green plants. Each of these dietary pigments typically produces a yellow or orangish-yellow hue when deposited in integumentary structures such as skin, scales, bill sheaths, or feathers (Saks et al. 2003; Lim et al. 2024). Few animals ingest significant quantities of red carotenoids; therefore, most animals must oxidize yellow dietary carotenoids into red C4-ketocarotenoids to produce saturated red coloration (Brush 1990; Mcgraw 2006; Toomey et al. 2022). The oxidation of yellow precursor carotenoids to C4-ketocarotenoids lengthens the chain of double bonds with which photons interact and shifts the hue of the molecules toward red (Britton 1995). The enzymatic conversion of yellow dietary pigments to red pigments used in display is the central process determining the quality of red ornamentation (Alonso-Alvarez et al. 2022; Toomey et al. 2022). Moreover, in some species, as the rate and efficiency of carotenoid metabolism decreases, more yellow pigments are co-deposited with red pigments, causing a shift to less red (more orange) coloration (Hill et al. 2019; Hudon et al. 2025). Such a shift in coloration is likely to impact the reproductive success of males in species where females have mating preferences for males with saturated red coloration (reviewed in Hill 2006a).

A breakthrough in understanding the mechanism underlying the production of red coloration came with the discovery of cytochrome P450 2J19 (CYP2J19), a key enzyme that is required for production of red ketocarotenoid pigments in many birds and turtles. In studies of red-plumaged and yellow-plumaged Common Canaries (*Serinus canaria*) and red-billed and yellow-billed Zebra Finches (*Taeniopygia castanotis*), genetic changes at the *CYP2J19* locus were strongly associated with the color phenotype (Lopes et al. 2016; Mundy et al. 2016). In the canary, the version of *CYP2J19* that enables production of red carotenoids from yellow dietary pigments was introgressed from the Red Siskin (*Spinus cucullata*) into domestic Common Canaries via hybridization (Birkhead 2003). Researchers were then able to identify the introgressed siskin version of *CYP2J19* in the otherwise canary genetic background (Lopes et al. 2016). In the Zebra Finch, the yellow-billed condition, which was fixed in a breed of domestic birds, resulted from a single nucleotide polymorphism in *CYP2J19* that caused loss of function of its carotenoid-metabolizing enzyme product (Mundy et al. 2016).

When first discovered, CYP2J19 was hypothesized to catalyze the conversion of yellow dietary carotenoids such as lutein and zeaxanthin to red C4-ketocarotenoids such as α-doradexanthin and astaxanthin, thus serving as a stand-alone element necessary for production of red coloration in birds (Barsh 2016; Lopes et al. 2016; Mundy et al. 2016). However, when the

enzymatic activity of CYP2J19 was tested with yellow precursor carotenoid substrates, it did not catalyze the production of red C4-ketocarotenoids. Instead, CYP2J19 yielded modified yellow carotenoids that are not typical feather pigments in birds (Toomey et al. 2022). Investigation revealed that CYP2J19 was a necessary but not sufficient enzyme for production of red ketocarotenoids from yellow dietary precursors. A second enzyme, 3-hydroxybutyrate dehydrogenase 1-like (BDH1L), is also required for birds to convert yellow dietary carotenoids to red ketocarotenoids. BDH1L catalyzes further oxidation of the yellow intermediate pigments produced by CYP2J19 into the red C4-ketocarotenoids that are used to produce red integumentary displays (Toomey et al. 2022). Given the discovery of BDH1L and the observation that CYP2J19 alone does not catalyze the production of red pigments, any species in which CYP2J19 activity related to red pigmentation has been described is likely using the two-enzyme CYP2J19/BDH1L system.

Alternative pathways to red

The CYP2J19/BDH1L pathway appears to be the exclusive pathway used by birds to produce ketocarotenoids for colored oil droplets that are used as spectral filters in the retina and that are nearly ubiquitous in visual systems across Aves (Emerling 2018; Twyman et al. 2018). It was hypothesized—and supported in evolutionary reconstructions—that this pathway originally evolved to convert dietary yellow carotenoids to red ketocarotenoids in the retina to enhance color vision in vertebrates and that it was subsequently co-opted to produce red coloration in integumentary structures (Twyman et al. 2016). A pathway requiring CYP2J19 has been confirmed to be a route to red carotenoid coloration in several species of birds—as well as turtles—with red coloration (Twyman et al. 2018; Khalil et al. 2020; Kirschel et al. 2020; Aguillon et al. 2021; Hooper et al. 2024). The implication from these studies is that CYP2J19/BDH1L could be assumed to be the enzymatic pathway for production of red coloration in birds that use ketocarotenoids as colorants (e.g., Hill et al. 2019; Cantarero et al. 2020).

Questions regarding the ubiquity of the CYP2J19/BDH1L pathway arose in follow-up studies of the House Finch (*Haemorhous mexicanus*), the most intensively studied bird with red carotenoid coloration (Hill 2002a). Unlike species in which the CYP2J19/BDH1L pathway has been confirmed and that use astaxanthin or α-doradexanthin as primary red colorants (e.g., Khalil et al. 2020; Lindsay et al. 2022), male House Finches appear to use the dietary carotenoid β-cryptoxanthin to produce their primary red pigment, 3-hydroxy-echinenone (Inouye et al. 2001; McGraw et al. 2006). However, assays of CYP2J19 and BDH1L provided with β-cryptoxanthin as substrate did not produce 3-hydroxy-echinenone; rather, this substrate yielded astaxanthin and adonirubin (Koch et al. 2025). Moreover, this β-cryptoxanthin to 3-hydroxy-echinenone conversion involves the metabolism of yellow carotenoid substrates into asymmetric β,β-C4-ketocarotenoids, a reaction not typically observed in vertebrates (Koch et al. 2025). It is not a transformation that seems likely to occur via in the CYP2J19/BDH1L pathway, which has been found to consistently catalyze the oxidation of any available β-ring (Koch et al. 2025). Further research indicated that *CYP2J19* is not expressed in the tissues associated with ornamental red carotenoid metabolism in molting male House Finches, despite a fully functional CYP2J19/BDH1L system producing red pigments for color vision in House Finch retinas (Koch et al. 2025). The investigators concluded that CYP2J19 is unlikely to contribute to the production of red pigments used for ornamentation House Finches. Thus, within a single clade of songbirds (House Finch versus Red Siskin in family Fringillidae)—and even among different tissues within a single species (retinal tissue versus liver and follicle tissues in House Finches)—birds appear to utilize at least two distinct enzyme systems to produce red carotenoid-based coloration: the CYP2J19/BDH1L pathway and a pathway catalyzed by unknown enzymes that converts β-cryptoxanthin to 3-hydroxy-echinenone (Fig. 1).

At least one additional pathway to red carotenoid coloration has also been documented in passerine birds. Although it has not been confirmed with controlled feeding experiments matching precursors to products, there is strong evidence that some birds can endogenously produce the brilliant red/purple carotenoid rhodoxanthin from yellow dietary pigments using yet-to-be-discovered enzymes (Fig. 1). Some birds produce reddish coloration by directly ingesting the plant-produced carotenoid rhodoxanthin (e.g., Witmer 1996; Hudon and Mulvihill 2017), but there is substantial evidence that other birds produce rhodoxanthin from yellow dietary precursors. Such endogenous production of rhodoxanthin was first proposed by Hudon et al. (2007) in a study of the Pin-tailed Manakin (*Ilicura militaris*) and then supported in a broader survey of birds in family Pipridae (Hudon et al. 2012). Prum et al. (2012) also proposed that rhodoxanthin may be endogenously produced via the same or similar pathway in some Cotingas (family Cotingidae, the sister family to Pipridae) with red or purple feathers. Interestingly, among the 11 species of manakins with red color assessed in

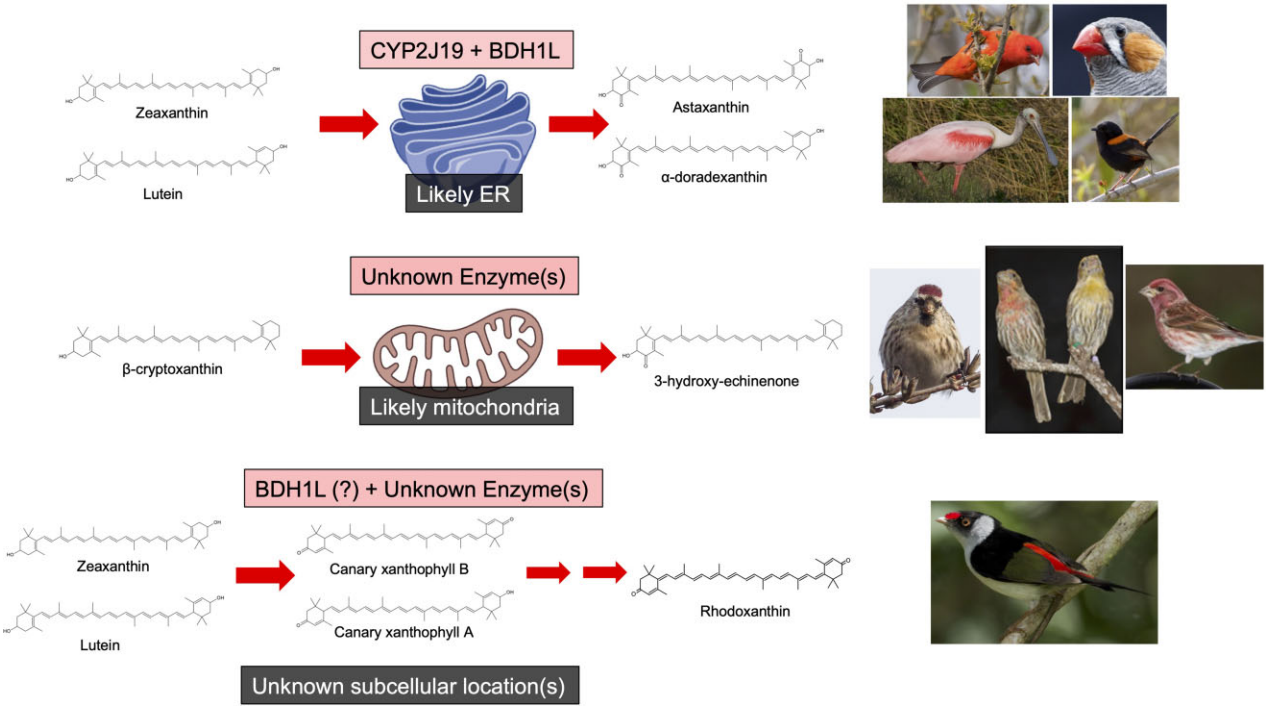

Fig. 1 Three pathways from yellow dietary carotenoids to ornamental red ketocarotenoid that current data support are used by birds to create red feather coloration. These pathways can create red color displays with comparable spectral characteristics, but they differ fundamentally in the biochemical transformations they catalyze and in their associations with subcellular compartments and cellular processes. The top pathway represents the CYP2J19/BDH1L pathway that is thought to produce many of the red ketocarotenoids across bird species, and uses a two-step enzymatic conversion to transform yellow dietary precursors, like zeaxanthin and lutein, into symmetric red β,β-C4-ketocarotenoids, like astaxanthin and α-doradexanthin (Toomey et al. 2022); these enzymes have been found to localize to the endomembrane system, likely the ER (Koch et al. 2025). The middle pathway represents the alternative pathway proposed to be used by species like the House Finch to convert yellow dietary precursors, like β-cryptoxanthin, into asymmetric red β,β-C4-ketocarotenoids, like 3-hydroxy-echinenone (Koch et al. 2025); studies of the House Finch suggest that this pathway occurs at least in part in the mitochondria (e.g., Hill et al. 2019). The bottom pathway represents the alternative pathway proposed to be used by species like manakins and cotingas to produce rhodoxanthin, a retrocarotenoid; BDH1L could be involved in an early step in this process, producing modified yellow canary xanthophylls from dietary precursors, but the enzymatic pathway itself to produce rhodoxanthin remains unknown (Hudon et al. 2007; Hudon et al. 2012; Prum et al. 2012). Birds pictured use the red carotenoid products specific to each pathway in their ornamental coloration. Top species, clockwise from top left: Scarlet Tanager (*Piranga olivacea*), Zebra Finch, Red-backed Fairywren (*Malurus melanocephalus*), and Roseate Spoonbill (*Platalea ajaja*). Middle species, from left: Common Redpoll, House Finch, and Purple Finch (*Haemorhous purpureus*). Bottom species: Pin-tailed Manakin (courtesy of João Vitor Perin Andriola).

Hudon et al. (2012), each species appeared to deposit either C4-ketocarotenoids consistent with the CYP2J19/BDH1L pathway, or the retrocarotenoid rhodoxanthin; this suggests that a subset of manakins have downregulated the CYP2J19/BDH1L system in favor of an alternative pathway that produces rhodoxanthin. Additionally, birds producing rhodoxanthin also had notable amounts of modified yellow ε,ε-carotenoids, like canary xanthophylls, which have been hypothesized to be the first step in the metabolic pathway leading from a dietary carotenoid to rhodoxanthin (Hudon et al. 2007). In the absence of CYP2J19, BDH1L catalyzes the metabolism of ε,ε-carotenoids like canary xanthophyll B from dietary β,β-carotenoids like zeaxanthin (Toomey et al. 2022). Consequently, it is plausible that BDH1L is involved in the beginning of the enzymatic pathway leading to the production of rhodoxanthin. House finches, which use a different alternative pathway to produce 3-hydroxy-echinenone, do express *BDH1L* in growing feather follicles and also have some canary xanthophylls in their feathers. Perhaps birds using either the rhodoxanthin pathway or the 3-hydroxy-echinenone pathway downregulate *CYP2J19* expression in tissues related to ornamental pigment production but maintain *BDH1L* expression.

Additional pathways to red coloration that do not involve carotenoid pigments exist in Class Aves, such as the recently discovered mechanisms responsible for the modification of endogenously produced psittacofulvin pigments to red forms in

parrots (Arbore et al. 2024), but the CYP2J19/BDH1L, 3-hydroxy-echinenone, and rhodoxanthin pathways are the best documented carotenoid-based pathways to date.

Consistent or variable hues

For nearly all species of birds that express species-typical red carotenoid coloration in the bill or plumage, all individuals of the displaying sex(es) and age class are red. Yellow variants are so rare that they can assumed to be loss-of-function mutations (McGraw et al. 2003; Hudon et al. 2007). When there is variation among males in these populations, it lies primarily in the saturation of the red pigmentation (Jones et al. 2010; Lindsay et al. 2011), although individuals in some red species also vary somewhat in hue (Kirschel et al. 2020). For this majority of red birds, variation in hue is small, and even extreme color variation does not produce a hue that a human would interpret as yellow. However, there are revealing exceptions to this color consistency.

In at least four bird species that use 3-hydroxy-echinenone as their primary red pigment (House Finch, Red Crossbill [*Loxia curvirostra*], White-winged Crossbill [*Loxia leucoptera*], and Pine Grosbeak [*Pinicola enucleator*]), yellow individuals are observed in most populations, sometimes comprising 25% or more of local populations (Hill 1993; del Val et al. 2014). This loss of red coloration has been shown to result not from a knockdown mutation but rather to be a result primarily of environmental conditions and individual health (Hill 2002). It is interesting that regular loss of red coloration seems to occur exclusively in species that use 3-hydroxy-echinenone as their primary red pigments and not in species that are known or likely to use the CYP2J19/BDH1L pathway, suggesting that variable color expression might be a characteristic of the pathway leading to 3-hydroxy-echinenone. However, individuals in many species that use 3-hydroxy-echinenone as their primary red pigment are as consistent in expression of red pigmentation as birds that use the CYP2J19/BDH1L pathway (e.g., Common Redpoll, *Acanthis flammea*, [Seutin et al. 1992]; Scarlet Rosefinch, *Carpodacus erythrinus*, [Albrecht et al. 2009]). One explanation is that there are variations in the pathway(s) from dietary yellow pigments to 3-hydroxy-echinenone, with one resulting in variable expression of redness while the other does not. Alternatively, individuals in these variable species might carry a high mutational load, leading to more frequent metabolic dysfunction and loss of coloration, or perhaps the fact that 3-hydroxy-echinenone (but not other common ornamental ketocarotenoids, like astaxanthin) can be readily converted to vitamin A could affect pigment use (Hill and Johnson 2012; Koch et al. 2025). However, these explanations are purely speculative, and additional study is needed to fully understand why some species commonly show yellow-to-red variation while many others do not.

Carotenoid metabolism and mitochondrial respiration

As reviewed above, the red carotenoid pigments used as integumentary colorants by vertebrates are, with few exceptions, ketocarotenoids; therefore, the conversion of yellow dietary carotenoids to red ketocarotenoids requires transformation via oxidation reactions (Prager et al. 2009; Prum et al. 2012; Cantarero et al. 2025). Better understanding these metabolic conversions has opened up new avenues for exploring how the mechanisms of producing carotenoid-based coloration may relate to the use of these ornaments as honest signals of quality. For example, it has been proposed that red ketocarotenoid production might occur within or in close association with the mitochondrion because the packaging of lipids and lipid-like molecules often occurs in the mitochondrion (Hill and Johnson 2012), and the inner mitochondrial membrane presents a redox environment that should be conducive to the sort of oxidation reactions that produce ketocarotenoids (Johnson and Hill 2013). Such an association between the production of red pigments and mitochondria is intriguing because it might explain the links between the red coloration and aspects of individual quality that may also be sensitive to mitochondrial performance, such as cognition, immune function, and oxidative stress (Hill and Johnson 2012; Johnson and Hill 2013; Hill 2014). If the production of red pigments relies on mitochondrial state, and mitochondria are also involved in a number of central systems and pathways that contribute to an individual's overall quality, then this mechanistic relationship could be key toward explaining honest signaling in red carotenoid-based ornaments (Koch et al. 2017).

As a first test for an association between mitochondria and the production of red pigments, Ge et al. (2015) isolated mitochondria from liver cells of molting male House Finches using centrifugation and tested the mitochondrial pellets for presence of carotenoids. These authors proposed that detection of ketocarotenoids within the mitochondrion would be empirical support for a link between production of red pigments and mitochondrial function. As predicted, unlike the typical brown coloration of mitochondria isolated from most vertebrates, the mitochondrial pellets isolated from the livers of molting male House Finches were bright red. Carotenoid analysis revealed high concentrations of ketocarotenoids in the final mitochondrial pellet (Ge

et al. 2015). However, the pellets at the end of this isolation process were not pure mitochondria; they contained both mitochondria and membranes bound to the mitochondria. Thus, from this study, it was not possible to confirm that ketocarotenoids were located within the mitochondria of House Finches, as opposed to being located in membranes adjacent to mitochondria (Ge et al. 2015). In a follow-up study, researchers took mitochondrial isolation a step farther, using high speed centrifugation to shear off the outer mitochondrial membrane, leaving a pellet comprising primarily the inner mitochondrial membrane and mitochondrial matrix extracted from molting male House Finch livers (Hill et al. 2019). Compared to membranes that were spun away, the pellet of inner mitochondrial membrane and matrix had high concentrations of ketocarotenoids (Hill et al. 2019). These observations support the hypothesis that red carotenoid metabolism in House Finches takes place in the inner mitochondrial membrane, in close association with the electron transport chain of aerobic cellular respiration.

Locating the metabolism of red carotenoids within mitochondria of House Finches suggested a functional association between mitochondrial respiration and carotenoid oxidation, but a direct test of this hypothesis was needed. The initial test of the hypothesis that carotenoid metabolism and color expression are dependent on mitochondrial performance was a simple correlational analysis conducted with wild House Finches. Hill et al. (2019) measured the hue of growing feathers for a sample of wild male House Finches and then quantified different metrics of mitochondrial performance and turnover rate in those same birds. They found that males growing feathers with redder hues, which is a reflection of higher concentration of red ketocarotenoids, had higher respiratory control ratios for state 1 precursors (a common measure related to mitochondrial respiratory efficiency), high inner mitochondrial membrane potentials (indicating efficient oxidation phosphorylation, or OXPHOS, processes), and lower PGC-1α (indicating less turnover of mitochondria) (Hill et al. 2019). These observations bolstered the hypothesis that red carotenoid metabolism is closely tied to mitochondria such that high quality red carotenoid ornamentation depends on high performing mitochondria.

In a follow-up study, Koch et al. (2024) again studied the association between mitochondrial function and plumage redness in male House Finches, but rather than relying on natural variation among wild males, they experimentally induced an environmental challenge in one set of birds by holding them in cages during molt and compared these captive males to free-living males. Captive male House Finches had altered mitochondrial performance compared to free-living males, and they produced fewer red ketocarotenoids from yellow precursors, as measured in circulating carotenoid concentrations; importantly, both captive and free-living birds circulated the same concentrations of yellow precursors, indicating that the difference in ketocarotenoid levels was due to differences in metabolism rather than carotenoid access. Moreover, captive birds that maintained the highest respiratory control ratios in complex 2 of the electron transport chain despite challenge of being confined to cages also produced the most red ketocarotenoids (Koch et al. 2024), consistent with the connections between red pigmentation and mitochondrial metrics that had been found in wild House Finches (Hill et al. 2019).

These studies on carotenoids in mitochondria and associations between mitochondrial function and red carotenoid accumulation provide experimental evidence in support of the hypothesis that red ketocarotenoid metabolism occurs in the mitochondria, likely in close association with the electron transport system itself. The discovery of CYP2J19 seemed to crack open a key black box in our understanding of red ketocarotenoid production, identifying a carotenoid-metabolizing enzyme that could plausibly act within the inner mitochondrial membrane (Hill et al. 2019). However, the experiments linking mitochondria to red coloration were conducted with the House Finch, which has now been shown not to use CYP2J19 to produce its ornamental red pigments (Koch et al. 2025). Moreover, experiments tracking the subcellular localizations of CYP2J19 and BDH1L have revealed that neither of these enzymes localizes to the mitochondria; instead, both appear to co-localize to the endomembrane system, likely the endoplasmic reticulum (ER) itself (Koch et al. 2025). Further supporting a fundamental difference between carotenoids metabolized via the CYP2J19/BDH1L pathway and the pathway leading to 3-hydroxy-echinenone, the mitochondrial pellets of molting male Northern Cardinals, a species that likely utilizes the CYP2J19/BDH1L pathway to produce red feathers (Sin et al. 2020), are brown rather than red and have only a trace of red ketocarotenoids (unpublished observations). The cellular location of CYP2J19/BDH1L and the lack of red pigments within the hepatic mitochondria of at least one species of bird that uses this pathway create uncertainty about whether red coloration derived from the CYP2J19/BDH1L pathway will be associated with mitochondrial performance.

This series of discoveries collectively paint a new picture of how, where, and why ornamental ketocarotenoid metabolism is occurring, and how these processes may differ among taxa. On the one hand, we have birds that have red coloration used as a signal

in mate choice and that produce red ketocarotenoids using the CYP2J19/BDH1L enzymatic pathway. In these species, such as the Northern Cardinal, the metabolism of carotenoids is predicted to take place within the ER, not in the mitochondria—and accordingly, isolated mitochondria from these species do not appear to contain carotenoid pigments. On the other hand, we have birds such as the House Finch, which has a variable red ketocarotenoid ornament, high levels of red ketocarotenoids associated with the inner membrane of hepatic mitochondria, and links between mitochondrial performance and production of red carotenoid carotenoids. The enzymatic system that produces ornamental red pigmentation in this latter group of birds is unknown. Thus, we have the most extensive understanding of the connections between mitochondrial function and red ketocarotenoid coloration in the system for which the enzyme(s) catalyzing the production of red ketocarotenoids from yellow precursors is unknown. Conversely, we have the best understanding of the enzyme systems that produce red carotenoid coloration for species in which physiology of color expression is less well characterized.

Effects of chemical treatments targeting mitochondria on red color expression

Potential differences between birds using the CYP2J19/BDH1L system and the alternative pathway that produces 3-hydroxy-echinenone are currently best understood from studies that experimentally alter mitochondrial states using chemical treatments. For example, Cantarero and Alonso-Alvarez (2017) tested the hypothesis that production of red carotenoids is linked to mitochondria by using chemical exposure to alter the redox environment of the inner mitochondrial membrane of Zebra Finches (family Estrildidae), a species that is confirmed to use the CYP2J19/BDH1L pathway (Mundy et al. 2016). They exposed Zebra Finches to one of two chemicals: either mitoquinone mesylate (mitoQ), a synthetic ubiquinone bound to decyl-triphenylphosphonium ($dTPP^+$) that localizes to the inner mitochondrial membrane and that can attenuate lipid peroxidation and maintain mitochondrial redox environment (Mao et al. 2022); or, $dTPP^+$ alone, which may instead decrease mitochondrial membrane potential (Trnka et al. 2015). They found that at the highest doses, mitoQ enhanced bill coloration relative to controls, while $dTPP^+$ caused a loss of redness (Cantarero and Alonso-Alvarez 2017). One explanation for these results is that, at high doses, an antioxidant effect of the ubiquinone portion of mitoQ counteracted the effect of decreased mitochondrial membrane potential by $dTPP^+$ and increased rate of red carotenoid metabolism, providing some of the strongest support to date for a link between mitochondrial dynamics and production of red pigments used for ornamentation in a species using the CYP2J19/BDH1L system. With the discovery that both of these enzymes localize to the ER rather than the mitochondria (Koch et al. 2025), the physiological relationship between mitochondrial performance and ketocarotenoid production in species like the Zebra Finch must be indirect, and requires further characterization.

Similar experiments have been conducted with the Red Crossbill, a Fringillid finch that, like the House Finch, deposits primarily 3-hydroxy-echinenone as its ornamental red pigment and that likely uses the same alternative to the CYP2J19/BDH1L pigment pathway (Koch et al. 2025). In one such experiment, Cantarero et al. (2020) dosed molting birds with either mitoQ or mitoTEMPO; mitoTEMPO is a synthetic antioxidant (a superoxide dismutase mimic) that is also delivered to the inner mitochondrial membrane and has been thought to have fewer potential negative side-effects than mitoQ (Cantarero et al. 2020). They found that mitoQ reduced circulating dietary carotenoids in the blood but did not change the feather redness of birds (Cantarero et al. 2020). The effects of mitoTEMPO treatment in crossbills appear to depend on physiological state; increased circulating red ketocarotenoid levels were observed in individuals that had less-red plumage at the time of capture from the wild, while increased plumage redness was observed only in individuals that had redder plumage at the time of capture (Cantarero et al. 2020). The authors considered the possibility that mitoTEMPO increased red ketocarotenoid production in all birds, but that the rate at which these carotenoids were metabolized and deposited in ornaments differed between high-quality (redder at start) and lower-quality (paler at start) individuals.

Collectively, results of studies using chemical manipulations demonstrate that changing mitochondrial parameters can change production of red pigments in birds that use either CYP2J19/BDH1L or an alternative enzymatic pathway for ketocarotenoid metabolism. Understanding the specific effects of treatment in these experiments will require better understanding of carotenoid metabolism in relation to subcellular and cellular function. If the production of ketocarotenoids is occurring in association with the ER in birds using the CYP2J19/BDH1L system, then connections between the ER and the mitochondria may explain how ketocarotenoid levels in such species remain sensitive to mitochondrial changes.

Concluding thoughts

Carotenoid coloration has long been viewed by researchers interested in color signaling as a single ornamental trait to which single explanations of evolution and function can be applied (e.g., Hill 2002; Blount and McGraw 2008; Willink and Wu 2023). There has been some consideration of fundamental differences in physiological control and signaling functions of carotenoid displays arising from unmetabolized dietary pigments versus metabolized pigments (Weaver et al. 2018) as well as from modified yellow versus red pigments (Hill and Johnson 2012; Delhey et al. 2023). Ornamental coloration arising from red carotenoids, however, has invariably been viewed as one trait (e.g., Prager and Andersson 2010; Powers and Hill 2021), even as multiple enzymatic pathways to red carotenoid coloration were hypothesized (Mcgraw 2006; Prum et al. 2012; Badyaev et al. 2015). Here, we highlight that different pathways that enable conversion of yellow dietary carotenoids to red pigments used in ornamentation are likely to have unique interactions with cellular processes and therefore to have different responses to environmental changes. Using insights from the study of and experimentation on birds using one enzymatic system to make predictions about coloration in other bird species that arise through an alternative enzymatic system is likely to result in confusion and to slow progress in fundamental understanding of color signaling. We propose that the most productive path forward is to expand understanding of the enzymatic pathways used by birds to produce red coloration and to then conduct studies that compare and contrast whether and how condition dependency arises from these distinct mechanisms of ornamental coloration.

Key unanswered questions

- Why do different bird species employ different enzymatic pathways from yellow to red carotenoids? What is the benefit of these innovations?
- Do associations between mitochondria and production of red pigments, documented in the House Finch system, extrapolate to species that derive red coloration using the CYP2J19/BDH1L system?
- Is the House Finch a model for all species that produce red feather coloration using asymmetric β,β-C4-ketocarotenoids?
- What are the implications for honest signaling of the CYP2J19/BDH1L system localizing in the ER?
- Why are some birds with ketocarotenoid-colored ornaments highly variable in coloration with frequent yellow individuals, while most species are not?

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Conflicts of interest

The authors declare no conflicts of interest related to research reported.

Data availability

No datasets were generated or analyzed during the current study.

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