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Invited Review

Avian Coloration Genetics: Recent Advances and Emerging Questions

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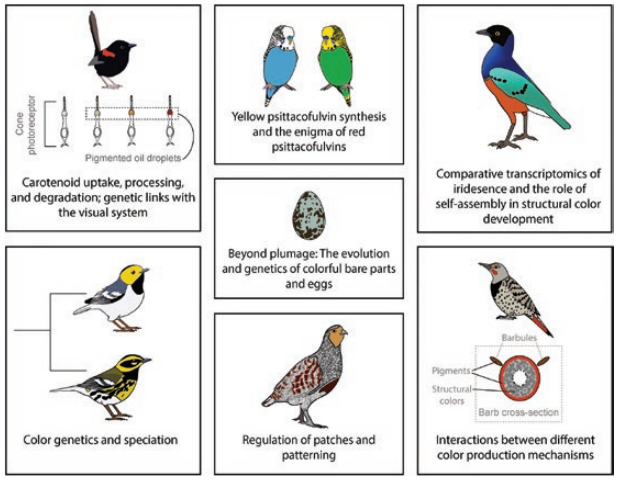
Abstract

The colorful phenotypes of birds have long provided rich source material for evolutionary biologists. Avian plumage, beaks, skin, and eggs—which exhibit a stunning range of cryptic and conspicuous forms—inspired early work on adaptive coloration. More recently, avian color has fueled discoveries on the physiological, developmental, and—increasingly—genetic mechanisms responsible for phenotypic variation. The relative ease with which avian color traits can be quantified has made birds an attractive system for uncovering links between phenotype and genotype. Accordingly, the field of avian coloration genetics is burgeoning. In this review, we highlight recent advances and emerging questions associated with the genetic underpinnings of bird color. We start by describing breakthroughs related to 2 pigment classes: carotenoids that produce red, yellow, and orange in most birds and psittacofulvins that produce similar colors in parrots. We then discuss structural colors, which are produced by the interaction of light with nanoscale materials and greatly extend the plumage palette. Structural color genetics remain understudied—but this paradigm is changing. We next explore how colors that arise from interactions among pigmentary and structural mechanisms may be controlled by genes that are co-expressed or co-regulated. We also identify opportunities to investigate genes mediating within-feather micropatterning and the coloration of bare parts and eggs. We conclude by spotlighting 2 research areas—mechanistic links between color vision and color production, and speciation—that have been invigorated by genetic insights, a trend likely to continue as new genomic approaches are applied to non-model species.

Graphical Abstract



Subject Area: Genotype to Phenotype
Key words: Carotenoids, Genotype to phenotype, Molecular adaptation and selection, Psittacofulvins, Self-assembly, Structural color

Birds have some of the most striking colors and patterns in nature. As vibrant as avian colors appear to humans, they likely look even more impressive to birds themselves: birds have tetrachromatic (4 color cone type) color vision that extends into the ultraviolet range (Vorobyev et al. 1998; Hart and Hunt 2007). Avian colors have wide-ranging functions as signals associated with courtship and mate choice, predator avoidance, social behavior, parent–offspring communication, and recognition (of species, mates, kin, and individuals). Beyond signaling, avian colors are often associated with photoprotection, thermoregulation, bacterial resistance, and structural support (Cuthill et al. 2017). The vast diversity of colorful avian phenotypes makes birds a powerful system for identifying the genetic and developmental bases of coloration, which is critical for understanding the origins of adaptive variation (Hubbard et al. 2010; Orteu and Jiggins 2020).

Colors in birds—their feathers, bare parts like skin and bills, and eggs—are produced by a variety of pigmentary and structural mechanisms (Figure 1). Of these mechanisms, melanin pigmentation has been

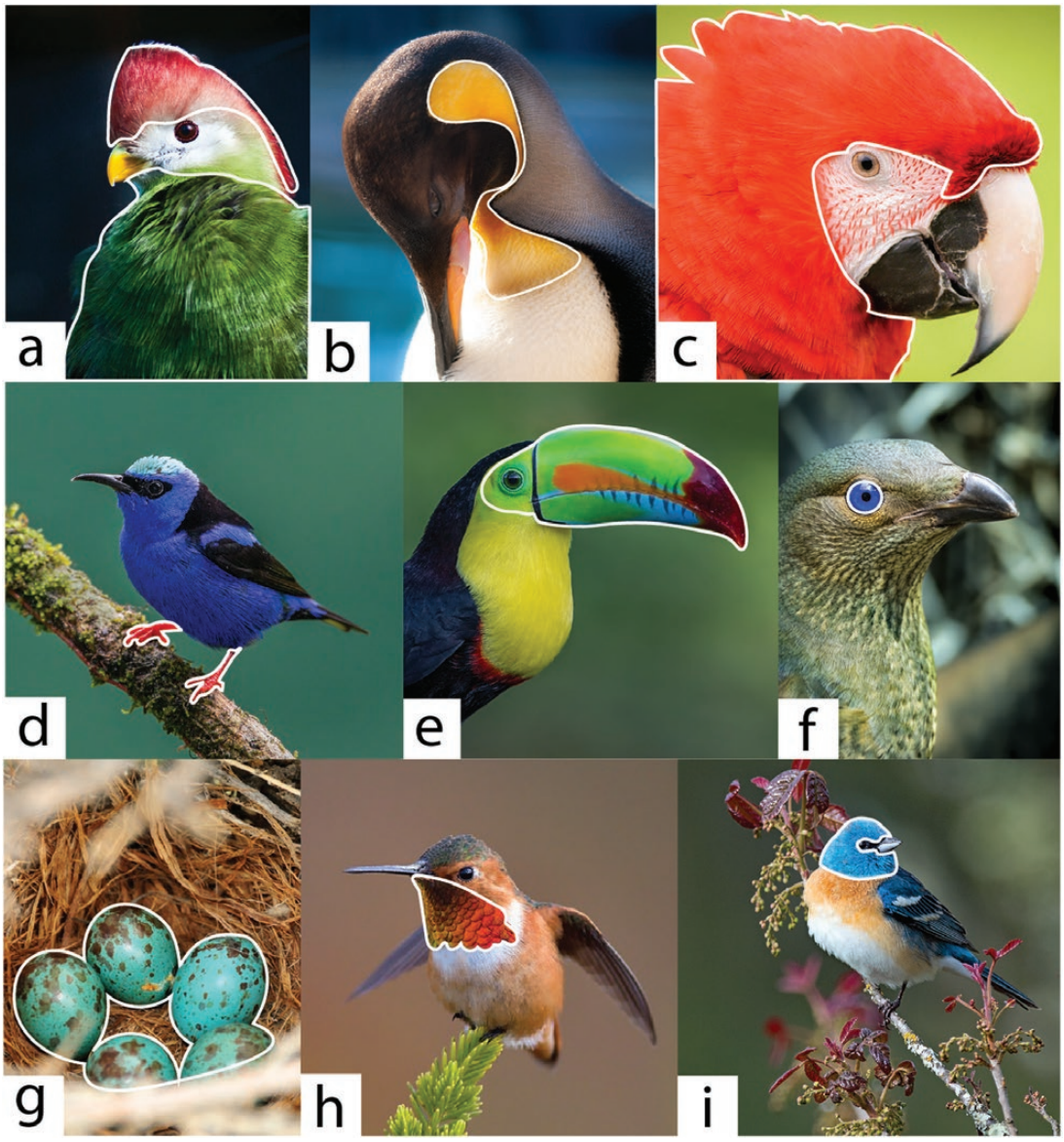


Figure 1. Emerging areas of research and ongoing mysteries in avian color genetics include uncommon pigments (**A**–**C**), coloration of bare parts and eggs (**D**–**G**), and structural colors in plumage (**H, I**). (**A**) Red turacin and green turacoverdin (porphyrin) pigments in a red-crested turaco (*Tauraco erythrolophus*). (**B**) Yellow sphenescin (pterin-like) pigments in a king penguin (*Aptenodytes patagonicus*). (**C**) Red psittacofulvin pigments in a scarlet macaw (*Ara macao*). (**D**) Red legs in a red-legged honeycreeper (*Cyanerpes cyaneus*). (**E**) Multicolored bill and eye ring in a keel-billed toucan (*Ramphastos sulfuratus*). (**F**) Blue iris in a satin bowerbird (*Ptilonorhynchus violaceaus*). (**G**) Blue speckled eggs of a sage thrasher (*Oreoscoptes montanus*). (**H**) Iridescent gorget in an Allen's hummingbird (*Selasphorus sasin*). (**I**) Blue head in a lazuli bunting (*Passerina amoena*). Relevant phenotypes are outlined in white. Photo credits: (**A**) Lindsay Wilson (CC BY 2.0), (**B**) Willian Warby (CC BY 2.0), (**C**) Pai Shih (CC BY 2.0), (**D, E**) Andy Morfew (CC BY 2.0), (**F**) Wade Tregaskis (CC BY-NC 2.0), (**G**) USFWS Mountain-Prairie (CC-BY 2.0), (**H**) Andrej Chudy (CC BY-NC-SA 2.0), (**I**) Doug Greenberg (CC BY-NC 2.0).

the focus of most research on coloration genetics in birds and other vertebrates. Early studies in birds used candidate gene approaches and focused on coding variation to identify a key set of genes involved in melanin pigmentation, particularly melanocortin-1 receptor (*MC1R*), agouti-signaling protein (*ASIP*), tyrosinase (*TYR*), and others (reviewed in Mundy 2005; Hubbard et al. 2010; Roulin and Ducrest 2013). More recently, high-throughput sequencing and other technological and computational advances have propelled coloration genetics research beyond candidate gene approaches and beyond melanin, especially in non-model and wild systems (San-Jose and Roulin 2017; Funk and Taylor 2019; Orteu and Jiggins 2020). These include whole-genome approaches, reduced-representation sequencing, sequence capture methods, and RNA sequencing (reviewed in Toews et al. 2016a). For example, in wild avian systems with low amounts of background genetic variation (such as hybrid zones or closely related radiations), reduced-representation and whole-genome sequencing have facilitated the mapping of genomic variation to variation in color phenotypes (e.g., Toews et al. 2016b; Brelsford et al. 2017; Campagna et al. 2017; Abolins-Abols et al. 2018; Kim et al. 2019; Hooper et al. 2019; Knief et al. 2019; Kirschel et al. 2020; Aguillon et al. 2021; Semenov et al. 2021). Measuring differential gene expression in alternate phenotypes can help identify candidate genes for coloration (e.g., Gao et al. 2018; Zheng et al. 2020; Rubenstein et al. forthcoming), and more targeted gene expression can be used to link candidate genes with specific color phenotypes (e.g., Mundy et al. 2016; Cooke et al. 2017; Khalil et al. 2020). Comparing whole genomes across large swaths of avian diversity can reveal broad patterns of molecular evolution (signatures of selection, gene duplications, and pseudogenization) in genes known to be involved in pigmentation and visual perception (Borges et al. 2015; Emerling 2018; Twyman et al. 2018a; Feng et al. 2020). Similar approaches can be used to identify additional candidate genes for coloration (e.g., Zhang et al. 2014; Gao et al. 2018; Prost et al. 2019).

The application of genomic methods to the study of color in birds and other animals has yielded major insights into adaptive evolution, shedding light on the genetic architecture of color traits, the importance of development in constraining color evolution, and the roles of sexual and natural selection in the wild (reviewed in Orteu and Jiggins 2020). In birds, the breakthroughs described above have set the stage for rapid progress toward identifying the genetic bases of diverse color production mechanisms (Box 1). In this review, we describe recent advances and emerging questions in the genetics of pigmentary plumage color, structural plumage color, and colors produced by interacting mechanisms. Next, we explore the genetic underpinnings of plumage patterning and the genetics of bare part and egg coloration. We end by highlighting 2 growing areas of interest: unraveling the genetic links between the visual system and carotenoid coloration, and using coloration genetics research to understand speciation. Our overarching goal is to highlight substantial recent progress toward understanding the genetic bases of avian coloration—while also noting that much of avian color diversity remains unstudied or enigmatic from a genetic and developmental standpoint.

Box 1 Highlights

- Genes related to carotenoid uptake, processing, and degradation are involved in carotenoid coloration. Simple regulatory switches in these genes can explain sexual dichromatism in some taxa.
- Yellow psittacofulvin pigmentation in parrots likely evolved by co-opting a polyketide synthase gene.
- Both self-assembly and active genetic control are involved in the development of complex within-feather patterning and structural colors.
- Physical interactions between different color production mechanisms are a common and essential part of the avian color palette. The importance of these interactions is likely reflected at the genomic level.
- Although most research on avian coloration genetics has focused on plumage, recent progress has been made toward understanding the genetic bases of bare parts (exposed skin, bills, legs, irises) and eggs.
- The synthesis of red ketocarotenoids from yellow dietary carotenoids likely evolved in the context of oil droplet pigmentation in the retina before being co-opted to produce red body coloration.

The Genetics of Pigmentary Plumage Colors: Carotenoids and Psittacofulvins

Overview of Pigments in Birds

Pigments are molecules that selectively absorb some wavelengths of light while allowing others to be reflected. The most common color-producing pigments in birds and other vertebrates are melanins (McGraw 2006b), which include eumelanin (brown and black) and pheomelanin (yellow to reddish brown). Most feathers colored by melanin contain a mix of eumelanin and pheomelanin, with the color of the feather determined by the relative abundance of each pigment type (McGraw 2006b). Melanin pigments also play a crucial role in producing spatial patterns (e.g., stripes, spots, bars) in a wide range of animals, including birds (Hoekstra 2006; McGraw 2006b; Inaba and Chuong 2020). Beyond their function in signaling, melanin pigments are often associated with photoprotection, antioxidant capacity, mechanical strength, resistance to bacterial degradation, and thermoregulation (reviewed in McGraw 2006b; Galván and Solano 2016; San-Jose and Roulin 2018; McNamara et al. 2021).

Carotenoids comprise a second major class of avian pigments, yielding most orange, red, and yellow colors in bird feathers, bills, skin, and irises. Birds, like most other animals, must obtain carotenoids from dietary sources (McGraw 2006a): carotenoids are synthesized by diverse plants, bacteria, and fungi. To produce a range of orange, red, and yellow carotenoids, birds must process and metabolize yellow dietary carotenoids through ketolation (addition of a keto group) or dehydrogenation, or they must deposit dietary carotenoids directly into skin or feathers (McGraw 2006a). At least 39 different carotenoids have been identified in avian plumage, most of which are produced from a relatively small set of dietary carotenoids (e.g., lutein, zeaxanthin, beta-carotene, beta-cryptoxanthin; LaFountain et al. 2015). Dietary and metabolized carotenoids are linked through complex biochemical networks; the evolutionary diversification of carotenoid coloration is constrained by network parameters such as robustness (i.e., the number of dietary precursors that can be used to synthesize the same carotenoid), connectivity, and length of the enzymatic pathways involved (Badyaev et al. 2015; Morrison and Badyaev 2016, 2018; Badyaev et al. 2019). As with melanins, carotenoids can have non-signaling functions,

with some carotenoids essential for immune system function and vitamin A synthesis (Bendich and Olson 1989; von Lintig 2010; Hill and Johnson 2012). Carotenoids are widely hypothesized to be honest signals of quality or condition in birds, either because dietary limitations might impose a tradeoff between coloration and immune system function or oxidative stress reduction (Olson and Owens 1998; Weaver et al. 2017; Koch et al. 2019) or because carotenoid coloration can function as an index of metabolic health (Hill 2011, Hill and Johnson 2012; Johnson and Hill 2013; Biernaskie et al. 2014; Weaver et al. 2017; Cantarero et al. 2020a, 2020b). However, there are many open questions about links between carotenoid color and individual quality (Weaver et al. 2017; Koch et al. 2018; McCoy et al. 2020), and the role of carotenoids in honest signaling remains a source of debate (LaFountain et al. 2015).

Some groups of birds have evolved the capacity to synthesize and deposit novel pigments. Turacos (family *Musophagidae*) use the copper-based porphyrin pigments turacin and turacoverdin to create red and green plumage colors, respectively (Figure 1A) (McGraw 2006c). Similar copper-containing pigments exist in Northern jacanas (*Jacana spinosa*) and 2 partridge species (Dyck 1992). The orange, red, and yellow iris colors in some avian eyes are produced by pterin pigments (McGraw 2006c), and yellow fluorescent feathers in some penguins are produced by a unique pigment (spheniscin) that is chemically similar to orange and yellow pterins (Figure 1B) (McGraw et al. 2007; Thomas et al. 2013). Parrots—which lack carotenoids in their feathers—color their plumage with endogenous (internally synthesized) psittacofulvin pigments, which produce orange, red, and yellow colors (Figure 1C), while yellow plumage in at least one starling is produced by vitamin A (Galván et al. 2019). Finally, bird egg colors are derived from pigments (Figure 1G; see "Beyond Plumage: The Genetics of Bare Part and Egg Coloration" section).

Among the many pigments birds use to color their feathers—melanins, carotenoids, psittacofulvins, pterins, vitamin A, and porphyrins—the genetic basis of melanin coloration is particularly well studied (reviewed in Mundy 2005; Hubbard et al. 2010; Roulin and Ducrest 2013; Galván and Solano 2016; McNamara et al. 2021). Melanins are relatively easy to study: they are endogenously produced, fairly straightforward to characterize, and widespread in vertebrates, including humans, mammals, and birds (Galván and Solano 2016). In contrast, the genetic bases of uncommon pigments (porphyrins including turacoverdin and turacin, pterins, vitamin A) are completely unknown. Here, we focus on the genetics of carotenoids and psittacofulvins, 2 pigment groups on which there has been recent rapid progress.

Carotenoids

Identifying the genetic basis of carotenoid coloration has been challenging (Hubbard et al. 2010; Toews et al. 2017). Carotenoids must be taken up, transported, metabolized, and deposited; the complexity of carotenoid processing required to produce the range of carotenoids identified in birds suggests these steps likely require a large number of genes (Toews et al. 2017; Mason and Bowie 2020). Encouragingly, high-throughput sequencing and other technological advances have led to substantial recent progress in uncovering the genetic basis of carotenoid coloration in birds (reviewed in Toews et al. 2017; Funk and Taylor 2019). In particular, several classes of genes involved in carotenoid uptake, ketolation, and degradation have emerged as key players in carotenoid coloration (Toews et al. 2017): these are the scavenger receptors, ketolases, and beta-carotene oxygenases, respectively.

Scavenger receptors, protein receptors that recognize the lipoproteins transporting hydrophobic carotenoids, mediate cellular carotenoid uptake. Scavenger receptors are important for carotenoid coloration in salmonids, silkworms, and scallops (Toews et al. 2017); only recently was their role in bird coloration confirmed. For example, a mutation in the splice donor site in scavenger receptor B1 (*SCARB1*) causes feather color to change from wild-type yellow to white in common canaries (*Serinus canaria*) (Toomey et al. 2017). In addition, scavenger receptor class F member 2 (*SCARF2*) is associated with carotenoid-based throat color in hybridizing Audubon's (*Setophaga coronata auduboni*) and myrtle (*Setophaga coronata coronata*) warblers (Brelsford et al. 2017).

Carotenoid ketolases are enzymes that convert carotenoids to ketocarotenoids via an oxidation reaction that adds a ketone (or carbonyl) group. Cytochrome P450 ketolases appear to be responsible for transforming precursor dietary yellow carotenoids to red ketocarotenoids in birds with red feathers. In particular, cytochrome P450 *CYP2J19* was first linked to variation in red coloration in the feathers of domestic "red factor" canaries (*Spinus cucullatus* × *Se. canaria*) (Lopes et al. 2016) and the bills and legs of "yellowbeak" zebra finch mutants (*Taeniopygia guttata*) (Mundy et al. 2016). Since those initial studies, *CYP2J19* has been linked to variation in red ketocarotenoid coloration across several distantly related avian lineages, including yellow-shafted (*Colaptes auratus auratus*) and red-shafted flickers (*Colaptes auratus cafer*) (Aguillon et al. 2021), weaverbirds (Family Ploecidae) (Twyman et al. 2018b), long-tailed finches (*Poephila acuticauda*) (Hooper et al. 2019), red-fronted (*Pogoniulus pusillus*) and yellow-fronted (*Pogoniulus chrysoconus*) tinkerbirds, (Kirschel et al. 2020), and red-backed fairywrens (*Malarus melanocephalus*) (Khalil et al. 2020). In red-backed fairywrens, testosterone mediates plumage redness via expression of *CYP2J19* (Khalil et al. 2020). Red-backed fairywrens are cooperative breeders; males can either produce red/black ornamented plumage or female-like brown plumage. Ornamented males (those with red plumage) had higher concentrations of circulating ketocarotenoids and higher *CYP2J19* expression in the liver than females and unornamented males. Additionally, unornamented males implanted with testosterone had higher hepatic *CYP2J19* expression than control unornamental males. These findings support the hypothesis that testosterone levels related to life history modify expression of *CYP2J19* in the liver, increasing the concentration of circulating metabolized red ketocarotenoids in the blood plasma that can be deposited in feathers (Khalil et al. 2020). In species such as zebra finches, *CYP2J19* is expressed in the peripheral tissues where ketocarotenoids are deposited but not in the liver, suggesting that the anatomical site of ketolation varies in different lineages (Mundy et al. 2016; Twyman et al. 2016). The identification of *CYP2J19* as an important carotenoid ketolase has provided an opportunity to investigate whether ketocarotenoids reflect individual condition via their links with cellular respiration in the mitochondria (Hill et al. 2019; Cantarero et al. 2020a).

Beta-carotene oxygenases are involved in carotenoid breakdown. Both regulatory and protein-coding variation in the enzyme beta-carotene oxygenase 2 (*BCO2*), which cleaves colorful carotenoids into colorless apocarotenoids, are linked to carotenoid coloration in sheep, cows, and birds (reviewed in Toews et al. 2017). In birds, *BCO2* was first linked to carotenoid coloration in domestic chickens, where regulatory variation affects the extent of yellow

carotenoid pigmentation in the skin (Eriksson et al. 2008). Both coding and regulatory variation in *BCO2* have also been linked to carotenoid pigmentation in canaries (Gazda et al. 2020a, 2020b), golden-winged (*Vermivora chrysoptera*) and blue-winged (*Vermivora cyanoptera*) warblers (Toews et al. 2016b), warblers in the genus *Setophaga* (Baiz et al. 2020b), and Darwin's finches (Enbody et al. 2021). In *Setophaga* warblers, *BCO2* evolution is associated with interspecific differences in carotenoid coloration across the 36 species in the genus (Baiz et al. 2020b). *BCO2* may also influence carotenoid color differences between males and females of the same species. Recently, Gazda et al. (2020a) showed that upregulation of *BCO2* in female *mosaic* canaries (*Sp. cucullatus* × *Se. canaria*) mediates sexual dichromatism. *Mosaic* canaries are hybrids generated by crossing and backcrossing sexually dimorphic red siskins (*Sp. cucullatus*) and sexually monomorphic common canaries (*Se. canaria*) to produce a strain of birds that are genetically similar to common canaries except in genomic regions related to dichromatism (Chen 2020; Gazda et al. 2020a). By measuring *BCO2* expression in developing feather follicles, Gazda et al. (2020a) showed that upregulation of *BCO2* in female feathers results in carotenoid degradation and white feathers. *BCO2* expression also appears to be regulated by estrogen because nonreproductive females develop similar plumage to males. The role of *BCO2* expression in carotenoid dichromatism appears to extend to at least one other species, the European serin (*Serinus serinus*), but *BCO2* expression is uncorrelated with carotenoid dichromatism in the house finch (*Haemorhous mexicanus*) (Gazda et al. 2020a) and the red-billed quelea (*Quelea quelea*) (Walsh et al. 2012).

Psittacofulvins

Psittacofulvin pigments are found only in parrots, giving rise to a broad range of red, orange, and yellow plumage colors—similar to those produced by carotenoids in other birds (Stoddard and Prum 2011). Unlike carotenoids, psittacofulvins do not come from dietary sources but are instead synthesized endogenously. The genetic basis of psittacofulvin pigmentation was completely unknown until recently, when Cooke et al. (2017) identified a gene responsible for yellow psittacofulvin pigment in the domestic budgerigar (*Melopsittacus undulatus*). Wild-type budgerigars have yellow feathers colored by psittacofulvins and green feathers that are produced by a combination of yellow psittacofulvins and blue structural color. Budgerigars with the recessive Mendelian *blue* trait lack yellow pigmentation, so that feathers that would be yellow in the wild-type are white and feathers that would be green in the wild-type are blue. Cooke et al. (2017) first used genome-wide association to find associations between genetic variation and the *blue* phenotype. After narrowing candidate regions to a single divergence peak containing 11 predicted genes, they identified an uncharacterized polyketide synthase gene (*MuKPS*) that is highly expressed in regenerating feather follicles and has a single amino acid change that perfectly associates with the *blue* phenotype. To confirm the role of *MuKPS* in psittacofulvin pigmentation in budgerigars, they expressed *MuPKS* from both wild-type and *blue* budgerigars in yeast and found that wild-type but not *blue* alleles produce yellow pigment. Finally, they compared mRNA-seq data from wild-type and blue budgerigars to published datasets from crow and chicken feathers. This revealed that *MuKPS* was expressed hundreds to thousands of times higher in the budgerigar than its homologue in the chicken or crow, both of which lack psittacofulvin pigmentation. When the chicken homologue was expressed in yeast, yellow pigments were produced. This suggests that *MuKPS* has a conserved (and currently unknown) function across birds and that parrots co-opted this gene for yellow psittacofulvin synthesis through gene regulatory changes (Cooke et al. 2017; Mundy 2018).

While *MuKPS* is clearly involved in yellow psittacofulvin synthesis, the genetic basis of red psittacofulvin pigmentation remains unknown. This is partly because we do not know whether the absorbance spectra of red and yellow psittacofulvin pigments differ because they have different chemical structures (similar to the difference between red and yellow carotenoids) or as a result of interactions with feather keratin that alter pigment conformation. Early work on the chemical structure of psittacofulvins showed that red feathers across a wide range of parrot species contain the same 4 fully conjugated aldehydes of 14, 16, 18, and 20 carbon atom chains (Stradi et al. 2001; McGraw and Nogare 2005). Chain length is proportional to the wavelength of maximum absorbance. More recent work showed that yellow budgerigar feathers contain a mix of pigments that are not aldehydes and lack the C_{20} component, implying that the differences between red and yellow psittacofulvins are due to changes in pigment structure. In other words, changes in color from red to yellow are due to a lack of pigment with longest (C_{20}) conjugated carbon backbone, a different oxidation state at the end of the polyene acyl chain, or both (Cooke et al. 2017; Neves et al. 2020). Red psittacofulvins might then be synthesized from yellow psittacofulvins by reducing the terminal carboxylic acid to an aldehyde (Cooke et al. 2017; Mundy 2018) and/or by adding a C_{20} component. An as-of-yet undetermined reductase might be responsible for reducing yellow psittacofulvin fatty acids to red psittacofulvin aldehydes (Cooke et al. 2017; Mundy 2018). This is analogous to the change between yellow and red carotenoids, where the addition of a ketone group (ketolation) by *CYP2J19* modifies yellow dietary carotenoids into red ketocarotenoids.

An alternative (but not mutually exclusive) hypothesis is that the difference between red and yellow psittacofulvins stems at least partially from interactions between the pigment and feather keratin, which cause a conformational change in the pigment (Stradi et al. 2001; McGraw 2006c; Barnsley et al. 2018). Such pigment–protein interactions are widespread in animal coloration; for example, the carotenoid astaxanthin binds to proteins in the carapace of lobsters to form a pigment–protein complex called crustacyanin. This results in a shift in the absorbance properties of the pigment to longer wavelengths, so that the carapace appears blue instead of red as is typical of astaxanthin (Cianci et al. 2002). Denaturing of crustacyanin proteins explains why lobsters turn red after being boiled or dehydrated, illustrating that protein–pigment interactions can have powerful effects on coloration (Shawkey and D'Alba 2017). In birds, pigment–protein interactions can produce dramatically different colors in feathers with the same carotenoid in different species (Mendes-Pinto et al. 2012; Shawkey and D'Alba 2017). Intriguingly, psittacofulvins change color after being extracted from feathers (Stradi et al. 2001), and a single parrot feather can contain multiple pigments in a color gradient, unlike carotenoid-based feathers (Barnsley et al. 2018). These observations suggest that the range of colors produced by psittacofulvins may be due to interactions between psittacofulvins and feather keratin and not due to chemical modifications of pigments (Stradi et al. 2001; McGraw 2006c; Barnsley et al. 2018). To test this hypothesis, Barnsley et al. (2018) characterized the psittacofulvins underlying red and yellow regions of the same feather in a yellow-naped amazon (*Amazona auropalliata*). Using resonance Raman spectroscopy, they show that red (but not yellow)

barbs of the feather exhibit a vibrational frequency that is consistent with (but does not prove) the presence of pigment–protein bonds. Although these data are promising, they do not unequivocally resolve the question of how differences in psittacofulvin hues are generated. Is variation in psittacofulvin-based color the result of chemical modifications, conformational changes caused by interactions with feather keratin, or both? Deciphering the genetic basis of psittacofulvin pigmentation will first require a more complete understanding of how color differences in psittacofulvin-containing feathers are produced.

The Genetics of Structural Plumage Colors

Structural colors are produced by the interaction of light with nanoscale materials that vary in refractive index (keratin, melanin, and air in feathers; collagen in skin; Figure 2B–D). The plumage, skin, and irises of many bird species are structurally colored (reviewed in Prum 2006; Saranathan and Finet 2021). They can be iridescent, changing in hue based on viewing or illumination angle (Figures 1H and 2C), or non-iridescent, appearing the same from all angles (Figures 1I and 2B). Iridescent plumage colors are produced by coherent scattering of light from arrays of keratin, air, and melanin-containing organelles (melanosomes) located in feather barbules. Melanosomes come in 4 varieties—solid cylindrical, hollow cylindrical, solid flat, and hollow flat—and are arranged in single or multilayer stacks within feather keratin (Figure 2C) (Durrer 1986; Prum 2006; Maia et al. 2013; Nordén et al. 2019). Non-iridescent structural colors in feather barbs are produced by quasi-ordered arrays of spongy keratin and air (Figure 2B), generally divided into those that are formed from closely packed spherical air cavities or those that are formed from a tortuous network of air channels (Prum 2006; Saranathan et al. 2012). Most blue, violet, and ultraviolet (UV) feather colors are non-iridescent structural colors. Overall, structural colors greatly expand the range of possible bird feather colors and may have evolved in part to create signals distinct from the natural environment (Stoddard and Prum 2011).

Despite the outsize contribution of structural colors to avian color diversity, the genetic bases of structural colors in birds have remained almost completely unstudied. Structural colors are difficult to study for several reasons. First, morphological variation that produces meaningful changes in color is generally at the nanoscale, making it technologically difficult to observe and measure, yet quantifying phenotypic variation is often the key to mapping genetic variation through association studies (Thayer et al. 2020). Second, little is known about the development of the nanostructures that produce structural color in plumage, which has complicated efforts to explore the underlying genetic program (reviewed in Saranathan and Finet 2021). However, this paradigm is changing. In this section, we highlight recent efforts to unravel the developmental details of structurally colored feathers—an essential step for investigating the genetic bases of structural coloration. We then describe a new study that represents a first step toward identifying genes involved in iridescent feather production. Finally, we suggest several avenues for future work on the genetics of structural color.

How do structural colors form in bird feathers? Iridescent colors arise from highly specific nanoscale arrays in feather barbules (Figure 2C), suggesting that nanostructural development must be precisely controlled. Paradoxically, no active cellular control of melanosome placement has yet been documented in feathers (Durrer and Villiger 1967; Maia et al. 2012; Shawkey et al. 2015), suggesting that iridescent nanostructures form instead through self-assembly. One possibility is that melanosomes self-assemble via depletion attraction forces that pin melanosome cells to each other and to the edge

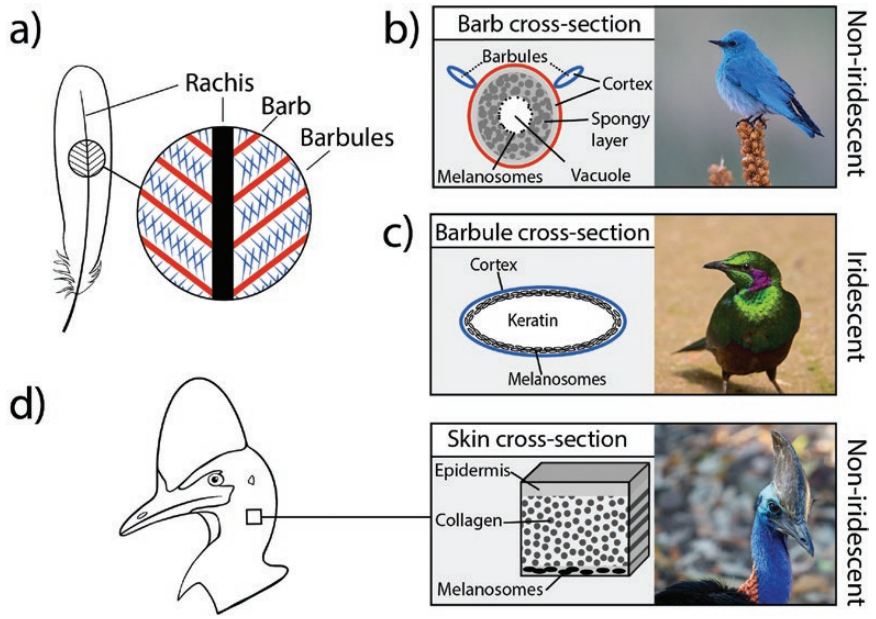


Figure 2. Mechanisms of structural color production in birds. (**A**) Schematic of feather showing hierarchical branching of barbs and barbules. (**B**) Non-iridescent structural color is produced by the keratinous spongy layer. (**C**) Iridescent structural color in feathers is produced by ordered arrays of melanosomes in keratin in feather barbules. Melanosomes shown are hollow and flat type, but can also be solid and flat, solid and cylindrical, or hollow and cylindrical. (**D**) Non-iridescent structural colors in bare parts (skin, podotheca, ramphotheca) are produced by collagen macrofibrils in a mucopolysaccharide matrix in the dermis. Macrofibrils are composed of smaller collagen fibrils (not shown). Birds shown are (**A**) Mountain bluebird (*Sialia currucoides*), (**B**) Emerald starling (*Lamprotornis iris*), and (**C**) Southern cassowary (*Casuarius casuarius*). Photo credits: (**A**) Andrej Chudy (CC BY-NC-SA 2.0), (**B**) Ian Morton (CC BY-NC-SA 2.0), (**C**) Jan Hazevoet (CC BY 2.0).

of the barbule cell (Box 2) (Maia et al. 2012). If this self-assembly process occurs in developing feather barbules, interactions of large melanosomes and small keratin particles could lead to thin-film or even hexagonally packed multilayer structures, a hypothesis consistent with morphological observations of iridescent feathers in blue-black grassquits (*Volatinia jacarina*) (Maia et al. 2012) and wild turkeys (*Meleagris gallopavo*) (Shawkey et al. 2015). The strength of depletion attraction may be affected by the size, shape, and concentration of melanosomes and by the concentration of keratin particles (Maia et al. 2012; Shawkey et al. 2015). Therefore, investigating the genetic bases of these traits may be critical for understanding the molecular bases of structural colors. For example, the melanosome scaffolding protein *PMEL* is known to affect melanosome shape (Hellström et al. 2011; McNamara et al. 2021) and might be expected to play a role in the diversification of melanosome shapes involved in iridescent structural colors. Similarly, iridescent barbules are often flattened relative to non-iridescent barbules (Doucet et al. 2006), and barbule shape may impact the strength of self-assembly within the developing barbule (Maia et al. 2012); the genes controlling barbule shape (which are mostly unknown, but see Chang et al. 2019) could also be involved in iridescent coloration. However, although the depletion attraction hypothesis provides clear predictions for how genetically encoded factors might impact self-assembly, it is not clear how depletion attraction can explain more complex, multilayered iridescent nanostructures seen in many birds (Maia et al. 2012; Saranathan and Finet 2021). Clearly, a better understanding of nanostructure development is necessary for guiding investigations into the molecular and genetic basis of iridescence.

As with iridescent colors, the developmental mechanisms underlying non-iridescent structural colors (Figure 2B) are thought to involve self-assembly (reviewed in Saranathan and Finet 2021). Specifically, spongy nanostructures in feather barbs are hypothesized to self-assemble through phase separation of β-keratin from the rest of the cytoplasm within the medullary cells (Box 2) (Dufresne et al.

Box 2 Self-assembly Processes in Avian Coloration

Depletion attraction dynamics (Asakura and Oosawa 1958) arise in mixtures of larger hard particles (attractants) and smaller polymers (depletants). The smaller depletants cannot invade a particular region surrounding the larger attractants. As the attractants approach each other, the volumes of the excluded regions surrounding each attractant overlap, which increases the volume that the depletant can occupy and reduces free energy in the system. The loss of free energy creates an osmotic gradient that pulls the attractants toward each other (Asakura and Oosawa 1958; Maia et al. 2012). Depletion attraction has been demonstrated or hypothesized in an increasing number of biological systems, where it may be involved in a range of cellular organization processes (Marenduzzo et al. 2006; Dorken et al. 2012; Sapir and Harries 2016; Zhao et al. 2019), including the formation of iridescent colloidal crystals in sporopollenin—the material in the tough outer coating of plant spores (Hemsley et al. 1994). In birds, depletion attraction forces are hypothesized to play a role in the formation of the keratin–melanosome nanostructures that produce **iridescence in feather barbules**. These nanostructures result from interactions between melanosomes (the attractants) and keratin particles (the depletants) during barbule keratinization (Maia et al. 2012; Shawkey et al. 2015), although other short-range forces may also be involved (Maia et al. 2012). The strength of depletion attraction is predicted to be affected by the shape, size, and concentration of melanosomes and keratin particles in barbules and by the shape of the barbule (Maia et al. 2012).

Phase separation is the spontaneous segregation of a mixture into 2 or more components. The role of phase separation is well established in the organization of cellular structures (reviewed in Hyman et al. 2014; Alberti 2017; Boeynaems et al. 2018; Yoshizawa et al. 2020) and formation of structurally colored materials (Zhao et al. 2012; Vohra et al. 2016; Shi et al. 2018). In birds, the keratin–air nanostructures that produce **non-iridescent structural colors in the medullary cells of feather barbs** are hypothesized to self-assemble through phase separation of β-keratin from cytoplasm (Dufresne et al. 2009; Prum et al. 2009; Parnell et al. 2015; Saranathan and Finet 2021). In particular, nanostructures may arise due to an incomplete phase separation process, where self-arrest of the process occurs as the result of cross-linking and competition between polymerizing β-keratin fibers (Dufresne et al. 2009; Saranathan and Finet 2021). Two different phase-separation mechanisms—spinoidal decomposition, and nucleation and growth—likely produce channel and sphere-type nanostructures in feather barbs, respectively (Dufresne et al. 2009).

Reaction-diffusion (Turing-like) mechanisms (Turing 1952; Kondo and Miura 2010) describe the formation of self-organizing spatial patterns as the result of the interplay between at least 2 interacting and diffusing factors, an activator and an inhibitor. Activators stimulate both their own production and the production of an inhibitor, but inhibitors diffuse at a longer range: the result is a heterogenous landscape of activator and inhibitor concentrations, with areas of high activator concentration surrounded by regions where inhibitors repress activator levels (Green and Sharpe 2015). Although initially controversial, theoretical and empirical work has now implicated reaction–diffusion in the production of a wide range of periodic patterns in animal coloration (reviewed in Kondo and Miura 2010; Green and Sharpe 2015; Neguer and Manceau 2017; Haupaix and Manceau 2020). In birds, reaction–diffusion dynamics likely explain complex within-feather **micropatterning** that arises as a result of the cyclical transfer of melanocytes to keratinocytes during feather growth (Prum and Williamson 2002). Reaction–diffusion involving various activators inhibited by bone morphogenic proteins (BMPs) is also involved in the spacing between feather follicles in the skin (Jung et al. 1998; Ho et al. 2019), resulting in complete inhibition of feather formation in chickens with elevated levels of BMPs (Mou et al. 2011). The local self-organization of patterns that results from reaction–diffusion is distinct from so-called positional or instructional models, in which the formation of patterns is spatially aware (reviewed in Green and Sharpe 2015; Haupaix and Manceau 2020).

2009; Prum et al. 2009). Consistent with these predictions, observations of spongy medullary cell development in blue-and-yellow macaws (*Ara ararauna*) indicate self-assembly through phase separation (Prum et al. 2009). Although no other study has examined the development of spongy arrays, Parnell et al. (2015) examined nanostructural variation across a single barb from a Eurasian jay (*Garrulus glandarius*) that varies in color from white, light blue, dark blue, and black. They showed that continuous variation in color is produced by spatial tuning in the degree of phase separation along the length of the barb. The degree of phase separation depends on interactions between polymerizing keratin fibrils, which may be impacted by the macromolecular properties of different β-keratins. Thus, research into the evolution and expression of β-keratin genes might reveal how variation in non-iridescent structural color is produced, a point raised recently by Saranathan and Finet (2021). Overall, self-assembly forces appear to be prominent in the development of structural colors in avian plumage (Box 2); understanding how self-assembly is involved in structural color development is an essential step for deciphering the molecular and genetic bases of structural colors. The challenge is to determine which developmental and genetic parameters set the stage for self-assembly (Maia et al. 2012).

Although the genetics of iridescence are virtually unexplored in birds, 2 studies have used comparative transcriptomics approaches to examine gene expression associated with iridescent feathers in comparison to non-iridescent feathers. Gao et al. (2018) compared the gene expression of pairs of feathers from golden (*Chrysolophus pictus*) and Lady Amherst's (*Chrysolophus amherstiae*) pheasants (e.g., iridescent green vs. white, yellow, or red feathers) and found that differentially expressed genes between non-iridescent and iridescent feather types were enriched in β-keratins. Rubenstein et al. (forthcoming) compared gene networks associated with developing iridescent blue feathers and non-iridescent reddish-brown feathers in the superb starling (*Lamprotornis superbus*). Consistent with Gao et al. (2018), they found that genes related to structural and cellular organization—including keratin genes—were upregulated in iridescent feathers, while genes related to pigmentation, metabolism, and mitochondrial function were upregulated in non-iridescent feathers. In particular, Rubenstein et al. highlight the upregulation of tyrosinase-related protein 1 (*TYRP1*) in iridescent feathers. Mutations in *TYRP1* have been linked to the formation of unusually shaped eumelanin-containing melanosomes in zebrafish (Braasch et al. 2009), chicken, and quail (Li et al. 2019), and—via interactions with *PMEL*—in mice (Hellström et al. 2011), suggesting *TYRP1* regulation may play a role in the formation of flattened and hollow melanosomes that produce iridescence in starlings (Rubenstein et al. forthcoming). Although these hypotheses require substantial further exploration, these comparative transcriptomics studies represent the first steps toward uncovering the molecular basis of iridescent color in birds.

There are several reasons to think that further progress in the genetics of avian structural coloration is imminent. First, careful characterization of the physical basis of structural color is essential for mapping genetic variation to phenotypic variation; approaches for quantifying nanostructural variation and modeling the physics of structural color are becoming more accessible and widely used (e.g., D'Alba et al. 2012; Wilts et al. 2014; Justyn 2017; Igic et al. 2018; Fan et al. 2019; Gruson et al. 2019; Bazzano et al. 2020; Eliason et al. 2020; McCoy et al. 2020). Captive budgerigars are an example of a particularly promising system for studying structural color genetics because the physical bases of pigmentary and structural color production in different morphs are well studied and detailed genetic data from breeders are available (D'Alba et al. 2012); this system was also the basis for groundbreaking research on psittacofulvin genetics (Cooke et al. 2017). Second, high-quality genomes and transcriptomes for species with both iridescent and non-iridescent structural color are becoming rapidly available and may prove useful in identifying candidate genes for structural color (Saranathan and Finet 2021). A dataset that included the genomes of 363 birds—including 267 newly sequenced genomes—was recently published (Feng et al. 2020). Such a treasure trove of genomic data may help pinpoint genes associated with structural color production, in the same way that whole-genome approaches have identified candidate genes for pigment synthesis (Zhang et al. 2014; Gao et al. 2018; Prost et al. 2019). That said, comparative genomics approaches are incomplete without corresponding applications of more direct methods for testing the roles of candidate genes in coloration (Saranathan and Finet 2021). Other approaches for uncovering structural color candidate genes might involve comparative transcriptomics or association studies in hybrid zones where hybrids have variation in structural color, similar to approaches that have been used to map variation in carotenoid-based color to hybrid phenotypes (e.g., Brelsford et al. 2017; Baiz et al. 2020a; Aguillon et al. 2021) or other closely related species with low levels of background genetic differentiation and clear differences in structural color. Few examples of disrupted structural color in hybrids have been described. One exception involves hybridization between the snow-capped (*Lepidothrix nattereri*) and opal-crowned (*Lepidothrix iris*) manakins, which leads to the formation of the hybrid golden-crowned (*Lepidothrix vilaboasi*) manakin species with nanostructural characteristics that are intermediate between the 2 parental species (Barrera-Guzmán et al. 2018). Finally, gene editing techniques are becoming more tractable in domestic avian systems (Dimitrov et al. 2016; Woodcock et al. 2017; Cooper et al. 2018) and can be used to directly link candidate genes to coloration. The application of gene editing techniques has recently led to breakthroughs in the genetics of structural colors in butterflies, revealing pleiotropic control of structural colors and pigments by *optix* (Zhang et al. 2017; Thayer et al. 2020) and *Wnt* genes (Fenner et al. 2020). Overall, the increasing sophistication of methods used to study both genomics and the physical bases of structural color suggests that breakthroughs in avian structural color genetics are at hand.

The Genetics of Plumage Colors Produced by Interacting Mechanisms

Interactions between coloration production mechanisms are a common and essential part of avian colors (Shawkey et al. 2009; Stoddard and Prum 2011; Shawkey and D'Alba 2017). Many so-called structural colors depend on interactions with pigments, and even so-called pigmentary colors may have a structural component via interactions with disordered nanostructures (reviewed in Shawkey and D'Alba 2017). These interactions are of critical importance to genetics research for 2 reasons. First, it is difficult to assign a particular color to any color production mechanism with certainty, and genetic variation in distinct color production pathways (i.e., either structural or pigmentary components) may result in meaningful phenotypic variation (Shawkey and D'Alba 2017; Fan et al. 2019). Second, the importance of multiple color production mechanisms for a given color phenotype means that we might expect

co-expression, co-regulation, or other genomic associations between distinct color production pathways. This phenomenon has recently been demonstrated in butterflies through shared regulation of pigments, structural colors, and other aspects of wing scale structure and patterning (Zhang et al. 2017; Matsuoka and Monteiro 2018; Fenner et al. 2020; Peng et al. 2020; Thayer et al. 2020; reviewed in Lloyd and Nadeau 2021). In this section, we review the importance of interactions between different color production mechanisms in shaping avian color phenotypes. We review one study in flickers that has demonstrated genomic associations between different color production mechanisms (melanins and carotenoids) and the same plumage patch. We also discuss how different coloration mechanisms might be co-regulated, using parrots as an example. Overall, we anticipate that associations between genes belonging to distinct coloration pathways with the same color trait are likely more common than is currently appreciated.

Different color production mechanisms interact in diverse ways. Most pigmentary colors are the result of color mixing from co-deposited pigments, including mixtures of co-deposited carotenoids (McGraw 2006a) and/or melanins (Jawor and Breitwisch 2003; McGraw 2006b). Combinations of structural colors and pigments are common and often produce colors that are not possible with either mechanism alone (Stoddard and Prum 2011; Shawkey and D'Alba 2017). For example, green plumage colors are often produced by the combination of structural colors produced by the spongy layer overlain by yellow pigments in the keratin cortex (Dyck 1971b; Prum 2006; D'alba et al. 2012). Although the prevailing wisdom is that blue structural and yellow pigmentary colors mix to produce green, this interaction is more complex than it initially appears. The structural color actually reflects both green and blue wavelengths: the final color appears green because yellow pigment absorbs the blue wavelengths and allows saturation of the green peak (D'Alba et al. 2012; Shawkey and D'Alba 2017). Pigments can also be deposited beneath the spongy layer (Figure 2B). For example, most non-iridescent structural colors contain a layer of melanin below the spongy layer which absorbs incoherently scattered light; without this pigment layer, the color of the structural color would be washed out (Prum 2006; Shawkey and Hill 2006; D'Alba et al. 2012; Shawkey and D'Alba 2017). Shifts between pheomelanin and eumelanin in the basal melanin layer can alter the appearance of structural colors from purple to blue (Peters et al. 2011; Fan et al. 2019). Carotenoids can also be deposited beneath the spongy layer (Dyck 1971a), although their effects on color appearance are less clear. Colors that are traditionally thought of as "pigmentary" may also have a structural component. For example, yellow plumage is produced by both the absorption of light by yellow carotenoid pigments and the reflection of light by an underlying array of disordered air spaces in keratin, so that the appearance of the carotenoid ornament critically depends on both the structural and pigmentary aspects of the feather (Shawkey and Hill 2005). Finally, different parts of the feather can also be colored by different coloration mechanisms (e.g., one pigment in barbs and another in barbules; or structural color in barbs and pigment in barbules). These interactions demonstrate that the distinctions between structural and pigmentary color are often blurry (Shawkey and D'Alba 2017). The same genes that are involved in pigmentation are likely to affect structural color (Hubbard et al. 2010; Saranathan and Finet 2021), and phenotypic variation in colors traditionally classified as non-iridescent structural colors may also be due to genetic variation in pigmentation pathways.

The interactions described so far demonstrate how colors can be produced by different color production mechanisms acting in concert (color mixing). Two alternative types of interactions—color masking and functional redundancy—can also affect color appearance. Color masking occurs when distinct color production mechanisms conceal one another. Pigments can obscure one another (Nero 1954; Moreau 1958; Hofmann et al. 2007); an example is the black malar stripe in yellow-shafted flicker (*C. a. auratus*) males, where red carotenoids are masked by melanin (Hudon et al. 2015; Aguillon et al. 2021). Melanin deposited above or within spongy layers can also mask structural color and produce black plumage (D'Alba et al. 2012; Kulp et al. 2018; Fan et al. 2019; Bazzano et al. 2020). Functional redundancy occurs when multiple mechanisms produce similar colors. For example, in bird plumage, similar long-wavelength colors can be produced by carotenoids, pheomelanin, some structural colors, and psittacofulvins (Stoddard and Prum 2011).

The importance of interactions—color mixing, color masking, or functional redundancy—among multiple color production mechanisms is likely reflected at the genomic level. One study in birds has identified associations between genes involved in distinct coloration pathways and the same trait, potentially as a result of color mixing or to avoid color masking. In their genome-wide association study of coloration in hybridizing yellow-shafted and red-shafted (*C. a. cafer*) flickers, Aguillon et al. (2021) identified a novel genetic link between known melanin genes and carotenoid traits (patches that display carotenoid coloration). Across the 7 plumage patches they examined, only one (the male malar stripe, discussed above) is known to contain both melanin and carotenoid pigments—yet they found repeated associations in multiple patches between known melanogenesis genes and carotenoid traits. They propose 3 non-mutually exclusive explanations: 1) the patches in question might have both melanin and carotenoids deposited in the feathers, with one pigment masking the other or mixing to produce the observed phenotype, 2) the genes involved in melanin pigmentation are pleiotropic, controlling aspects of both melanin and carotenoid pathways, and 3) the associations between melanin genes and carotenoid traits represent downregulation of melanin to prevent masking of carotenoid pigments by melanin. Future work could clarify these hypotheses through detailed physical and/or chemical examination of pigments from the feathers in question, as well as through gene expression studies in this system. The finding that regions of the genome associated with different color production mechanisms might be associated with the same region of plumage is novel from a genomics perspective, potentially because genes involved in multiple coloration pathways have rarely been studied in the same system. However, from a color production perspective, links between multiple coloration mechanisms and individual color traits are not unexpected. Given that interactions between color production mechanisms are common (Shawkey and D'Alba 2017), associations between genes involved in multiple coloration mechanisms and the same region of plumage may also be common.

The extent to which some avian lineages may have evolved to eliminate functional redundancy in color production is an open question: here, understanding whether and how different color production mechanisms are co-regulated is likely key. Parrots are an excellent group in which to explore this. The range of colors produced by parrot psittacofulvins overlaps with those produced by carotenoids (Stoddard and Prum 2011) and pheomelanin (Toral et al. 2008; Stoddard and Prum 2011; Galván and Wakamatsu 2016). Parrots have high levels of circulating carotenoids in blood and deposit carotenoid pigments in bare parts and in the retina, so their failure to deposit carotenoids in feathers is not due to a scarcity of carotenoids (McGraw and Nogare 2004). Intriguingly, parrots appear to deposit

eumelanin but not pheomelanin in feathers; this is the only known example of impairment of mixed melanin synthesis in birds (Neves et al. 2020). Preferential deposition of psittacofulvins over carotenoids and pheomelanin likely involves co-regulating carotenoid uptake genes, melanogenesis genes, and *MuKPS* or other psittacofulvin synthesis genes in feather follicles. Candidate genes for regulating the uptake of carotenoids in feathers versus in skin include scavenger receptors such as *SCARB1* (Toomey et al. 2017) or *SCARF2* (Brelsford et al. 2017). Neves et al. (2020) suggest that several genes—including *MC1R* antagonists *ASIP* and agouti-related proteins (*AGRP*)—may impair pheomelanin synthesis. Genes that control the bioavailability of cysteine, which is required for pheomelanin synthesis (Galván and Solano 2016; Galván 2018), are also likely candidates (Neves et al. 2020); these include the cysteine/glutamate antiporter xCT (*SLC7A11*) (Chintala et al. 2005) and cystinosin (*CTNS*) (Town et al. 1998). How carotenoid, melanin, and psittacofulvin pathways are co-regulated in parrots may be revealed through comparative transcriptomics of developing feather follicles, similar to approaches that have been used to identify candidate genes for pheomelanin pigmentation in chickens (Zheng et al. 2020) and iridescent plumage in superb starlings (Rubenstein et al. forthcoming).

Beyond complex interactions among color-producing nanostructures and pigments—and the effects of their location within the feather—feather microstructure (e.g., barb and barbule shape) can substantially alter the appearance of color produced by both pigments and nanostructures. Spiky and vertically oriented barbules in melanin-bearing feathers produce a deep, velvety-black plumage ("super black") that absorbs much more light than typical black feathers (McCoy et al. 2018; McCoy and Prum 2019). In iridescent feathers, changes in barbule shape can impact the angle dependence of iridescent colors by either increasing the range of hues produced (Wilts et al. 2014) or reducing iridescence (Dyck 1987; Harvey et al. 2013). In red and purple carotenoid-bearing feathers, barbs are often broadened and flattened and barbules are reduced (Brush and Seifried 1968; Olson 1970; Troy and Brush 1983; Badyaev et al. 2017; McCoy et al. 2020), increasing the perceived saturation (McCoy et al. 2020) and glossiness (Iskandar et al. 2016) of the color. A novel form of gloss was also recently described in cassowaries, produced by alterations of keratin in the feather rachis (Eliason and Clarke 2020). These examples demonstrate how selection on feather micro- and macrostructure can produce novel optical effects in birds, even without changes to the underlying pigment or nanostructure. The genetics of feather microstructures in the context of color production are unknown, but insights could come from work that has revealed how diverse feather morphologies have evolved through *cis*-regulatory changes in ancient morphogenesis signaling pathways (Prum 2005; Lowe et al. 2015; Seki et al. 2017; Ng and Li 2018; Chang et al. 2019) and from work showing developmental integration of carotenoid uptake and feather growth (Landeen and Badyaev 2012; Potticary et al. 2020).

The Genetics of Plumage Patterning

In addition to the remarkable diversity of colors produced by birds, complex plumage patterning is another striking feature of bird coloration (Stoddard and Osorio 2019; Mason and Bowie 2020). Patterns can include patterning across the body (the color and spatial arrangement of distinct plumage patches, sometimes referred to as "macropatterning") or patterning within a body region or feather (barring, striping, stippling, sometimes referred to as "micropatterning"; Inaba and Chuong 2020). Recent genetic advances have highlighted the extent to which plumage macropatterning evolves primarily as a result of changes in *cis*-regulatory regions, rather than through variation in coding regions (reviewed in Funk and Taylor 2019; Mason and Bowie 2020). Because the genetic regulation of plumage macropatterning has been recently reviewed (Funk and Taylor 2019; Mason and Bowie 2020), we do not focus on it here. Instead, we briefly summarize the developmental genetics of plumage micropatterning.

Regulatory changes can be spatial or temporal in nature. Changes in macropattern across the body are generally the result of spatial changes in expression: they alter the tissue-specific expression of color traits, resulting in changes in plumage patch color across different body regions separated by feather tracts. In contrast, changes in patterning along the length of a feather are the result of temporal changes in expression during development that alter the type, concentration, and distribution of pigments along the length of the feather, producing periodic micropatterns, such as stripes, spots, chevrons, and bars. The most complex patterns within feathers are produced by melanins (Prum and Williamson 2002; McGraw 2006b). Accordingly, feather micropatterns are often studied in the context of the transfer of melanosomes to keratinocytes during feather development. In contrast, carotenoids are deposited as a single contiguous patch within a feather, without any banding (McGraw 2006a). Micropatterns in birds and other vertebrates are generally thought to be the result of self-organizing reaction–diffusion mechanisms that cause the cyclical expression of activators and inhibitors that deposit pigments as part of a spatially constrained developmental process during feather growth (Box 2) (Turing 1952; Prum and Williamson 2002; Kondo and Miura 2010). As in the case of structural color, the balance between self-organization (reaction–diffusion models; Box 2) and active encoding of positional information (instructional models) of micropatterns presents an intriguing area of research for biologists (Haupaix and Manceau 2020). How are intricate periodic patterns with specific orientation and periodicity produced in a way that is replicable within species?

Understanding the mechanisms by which patterns are generated within feathers has been informed by a combination of genetic approaches, developmental biology, and mathematical biology, particularly within domesticated and model systems, such as chickens and Japanese quail (*Corturnix japonica*) (reviewed in Haupaix and Manceau 2020; Inaba and Chuong 2020). Recent work suggests that instructional and self-organizing mechanisms are linked to produce a range of periodic patterns. In birds and mammals, periodic patterns are often foreshadowed by "pre-patterns," or region-specific expression of genes that control the deposition of different types or amounts of pigments (Caro and Mallarino 2020; Haupaix and Manceau 2020). Across 10 galliform species, the direction and position of stripes are controlled by instructional developmental cues from the somatic mesoderm, which leads to stripe pre-pattern formation through differential regulation of *ASIP* (Haupaix et al. 2018). These early instructional cues are followed by self-organization via a reaction–diffusion mechanism that explains pattern periodicity and stripe width (Haupaix et al. 2018). Instructional cueing and self-organization are likely complementary mechanisms for pattern formation in birds and other vertebrates (Green and Sharpe 2015), with instructional cues that ensure reproducibility preceding self-assembly (reviewed in Haupaix and Manceau 2020). For example, the position and spacing of wing feathers likely involve both instructional cues from the Sonic Hedgehog (*Shh*) pathway followed by Turing-like self-organization (Pickering and Towers 2016;

Haupaix and Manceau 2020). Other recent insights into the molecular basis of plumage patterning have come from rock pigeons (*Columba livia*), a species where artificial selection has resulted in considerable variation in intricate patterning. Recent work in rock pigeons has linked copy number variation at the Stipper (*St*) locus to Almond patterning (Bruders et al. 2020), while both *cis*-regulatory and coding variation in Norrin (*NDP*) are linked to wing patterning (Vickrey et al. 2018).

Many questions remain about the genetic basis of plumage micropatterning, especially in wild birds. How do instructional cueing and self-organization produce the range of intricate periodic patterns seen across birds—and what are the precise identities of the activators and inhibitors involved in Turing-like self-organization (Box 2)? How important are regulatory changes, coding changes, and structural variants in generating evolutionary changes in plumage micropatterning? In general, much less is known about the genetic basis of complex patterning than about the specific types of melanins deposited in vertebrates (Hoekstra et al. 2006; Manceau et al. 2010; Mallarino et al. 2016), and plumage patterning remains one of the frontiers of avian coloration genetics.

Beyond Plumage: The Genetics of Bare Part and Egg Coloration

Although birds are perhaps best known for their elaborate plumage coloration, bare parts including unfeathered regions (exposed skin, feet, bills, eye rings), fleshy outgrowths (combs, wattles, snoods, tubercles, spurs, and caruncles), gapes (the inside of the mouth), and irises are often colorful (Figure 1D–F). In addition, avian eggshells can be extremely variable in color and patterning (Figure 1G). In spite of the taxonomic breadth and importance of bare part and egg coloration, studies of avian coloration have focused primarily on plumage coloration (reviewed in Iverson and Karubian 2017). The focus on plumage coloration extends to genetic studies. In this section, we briefly summarize the functions and mechanisms of bare part coloration. We summarize recent progress toward identifying the genetic bases of carotenoid coloration in bare parts and the genetics of iris color. We then discuss the functions and genetic underpinnings of egg coloration.

Colorful bare parts are taxonomically widespread in birds and often have important signaling and thermoregulatory functions (reviewed in Iverson and Karubian 2017). The signaling functions of colorful bare parts have been demonstrated in the contexts of competitive interactions and dominance (Emlen and Wrege 2004; Murphy et al. 2009; Dey et al. 2015), mate choice and assessment (Zuk et al. 1990; Velando et al. 2006; Simons and Verhulst 2011) and nestling signals to parents (Gótmark and Ahlström 1997; Kilner 1999; de Ayala et al. 2007; Wiebe and Slagsvold 2009) or between brood parasite young and their host parents (Hauber and Kilner 2007; Stoddard and Hauber 2017). The signaling functions of bare parts are likely distinct from those of feathers: while feather signals are relatively invariant within a season due to molting constraints, bare parts can be temporally dynamic and change in color or size rapidly. Unlike feathers, where patterning is often an important component of social signaling (Pollard and Blumstein 2011; Mason and Bowie 2020), bare parts generally lack patterning (Iverson and Karubian 2017). Bare parts are also under selection for functions outside of social and sexual signaling, such as thermoregulation (Burtt 1978; Negro et al. 2006; Ward et al. 2008; Stuart-Fox et al. 2017).

Like feathers, bare part coloration can be produced by pigments, structural coloration, or a combination; bare parts can also be colored by changes in blood flow under the skin, which can display or conceal colors (Negro et al. 2006; Iverson and Karubian 2017). However, several important differences exist between feather and bare part coloration. Among pigments, only carotenoids and melanins have been described from avian bare parts. Even in species that deposit uncommon pigments (psittacofulvins, pterins) in their feathers, these pigments do not appear to be deposited in bare parts (Iverson and Karubian 2017). Carotenoid coloration in bare parts is more taxonomically widespread than carotenoid coloration in feathers (Olson and Owens 2005). This may be because additional genes, such as those for binding carotenoid pigments to feather keratin, are needed for carotenoid deposition in feathers but not bare parts (Ligon et al. 2016; Iverson and Karubian 2017; Hill 2018). Some carotenoid pigments (e.g., carotenes) have been reported only from bare parts and not from feathers (McGraw 2006a). Carotenoids in bare parts also tend to be esterified (bound to fatty acids), which may improve pigment stability, particularly against photodegradation (McGraw 2006a; García-de Blas et al. 2013; Pérez-Rodríguez et al. 2016). The mechanistic basis of structural colors in avian skin and bills (Figure 1E) is distinct from that in feathers. In contrast to the keratin, air, and melanin-based structural colors in feathers, the structural colors in avian skin, bills, legs, and feet (all of which are non-iridescent) are produced by coherent scattering of light as it strikes 2-dimensional quasi-ordered arrays of parallel collagen fibers in the dermis, resulting in hues ranging from ultraviolet to yellow; some yellow and red skin is produced by a combination of structural color and carotenoid pigmentation (Prum and Torres 2003). Structurally colored skin ornaments (especially eye rings and fleshy facial ornaments) are widespread and likely evolved at least 50 times across birds (Prum and Torres 2003); identical collagen structures have convergently evolved to produce structurally colored skin in mammals (Prum and Torres 2004).

Another type of bare part is the avian iris, which is colored by mechanisms that are not present anywhere else in birds (Figure 1F; Prum 2006). Avian irises contain chromatophores, specialized pigment cells that contain crystalline purines (especially guanine), pteridines, or other pigments (Oehme 1969; Oliphant 1987; Oliphant and Hudon 1993; Hudon and Muir 1996; Prum 2006). Chromatophores are common components of integumentary coloration in fishes, amphibians, and reptiles but are found only in the irises of birds (Oliphant 1987; Oliphant et al. 1992; McGraw 2006c; Prum 2006). Iris colors can also be colored by carotenoids, melanins, or even hemoglobin, or a combination of multiple coloration mechanisms (McGraw 2006c; Prum 2006). Chromatophores may have been lost as skin colorants in birds and mammals following the evolution of feathers and hair, respectively, which presumably obscure chromatophore coloration in the dermis (Oliphant et al. 1992). Unlike other areas of a bird's feather-covered body, irises have continuously been exposed to the elements throughout evolution and may thus have been under sustained selection pressure for color signaling, retaining chromatophores as a result (Oliphant et al. 1992). A few studies show that iris color is likely an important social signal in many species (Craig and Hulley 2004) and may also be under selection for reduced conspicuousness in open-nesting and nocturnal groups (Davidson et al. 2017; Passarotto et al. 2018), but overall the evolution and function of avian iris color has been vastly understudied.

Studies of bare part coloration are much less common than feather coloration. However, many of the pioneering studies on

the genetic bases of carotenoid coloration focused on bare parts, including skin color in chickens (Eriksson et al. 2008; Yu et al. 2017), bill color in the red-bill quelea (*Quelea quelea*) (Walsh et al. 2012), and bill and tarsus color in zebra finches (Mundy et al. 2016). Two more recent studies have linked the *BCO2* to carotenoid coloration in bills: a mutation in *BCO2* results in carotenoid pigmentation of the bill in domestic urucum canaries (Gazda et al. 2020b), while a regulatory mutation in *BCO2* is responsible for nestling beak color polymorphism in Darwin's finches (Enbody et al. 2021). The evolutionary lability of carotenoid-based bare part coloration may thus be explained by simple switches in the regulation or enzymatic activity of *BCO2*. This scenario is similar to the upregulation of *BCO2* in female *mosaic* canary plumage, which provides a simple mechanism for the evolution of sexual dichromatism (Gazda et al. 2020a). As with plumage, most studies on the genetics of bare part coloration have focused on carotenoids and melanins. Two exciting exceptions are recent studies on the genetic basis of iris coloration in domestic pigeons (*Co. livia*). Mutant domestic pigeons with white "pearl" irises lack yellow pteridine pigments in their irises. Two studies independently showed that a nonsense mutation in the gene *SLC2A11B* is likely responsible for pearl-colored irises (Si et al. 2020; Andrade et al. 2021), consistent with its role in xanthophore (yellow chromatophore) differentiation in fishes (Kimura et al. 2014). Si et al. (2020) also identified a fixed frameshift mutation in *SLC2A11B* in several cormorant species, which have characteristically blue structurally colored irises that lack pteridine or purine pigments. The apparent link between *SLC2A11B* in pteridine synthesis in bird irises and in fish xanthophores suggests that birds may share a molecular mechanism for pteridine coloration with ectothermic animals (Andrade et al. 2021), consistent with the hypothesis that avian irises represent evolutionary refugia for chromatophores (Oliphant et al. 1992). Therefore, known pigmentation genes in fish may be good candidate genes for further investigation of iris coloration in birds (Andrade et al. 2021). Despite some progress on the genetics of carotenoid- and chromatophore-based bare part coloration, the genetic basis of the collagen fiber arrays that produce structurally colored skin, bills, feet, and legs is completely unknown. To our knowledge, collagen development and genetics have never been studied in the context of avian skin coloration. However, the molecular and genetic basis of collagen has been extensively studied in other systems (Kadler et al. 2008; Arseni et al. 2018; Holmes et al. 2018; Haq et al. 2019); insights into the regulation of collagen fiber diameter and spacing suggest promising avenues for research into the genetics of structurally colored bare parts in birds (Saranathan and Finet 2021). Overall, colorful bare parts represent a relatively underexplored area of avian evolutionary biology and genetics. Why is carotenoid coloration more widespread in bare parts than in feathers? Why are avian irises colored by mechanisms that are involved in no other aspects of avian coloration? Why do bare parts generally lack patterning? Further research on the genetics of bare part coloration may reveal answers to these questions.

No discussion of avian coloration would be complete without mentioning eggs, which range in color from white, beige, brown, or rusty red to blue, green, turquoise, purple, or pink. Eggshells can be immaculate or variably speckled, splotched, or squiggled (Hauber 2014). Although selection by predators for camouflaged eggs appears to be a potent driver of egg color variation (Stoddard et al. 2011), many other functions of egg coloration—including thermoregulation, sexual signaling, antimicrobial defense, identity signaling, mimicry by brood parasites, mechanical strength, and protection against solar radiation—have been proposed (Kilner 2006; Maurer et al. 2015; Lahti and Ardia 2016). Until recently, just 2 pigments—brown protoporphyrin and blue-green biliverdin (Figure 1G)—were believed to be responsible for the diverse array of eggshell colors in birds (Sparks 2011; Hanley et al. 2015). However, additional eggshell pigments have been discovered in tinamous (*Tinamidae*) (Hamchand et al. 2020), suggesting that the pigmentary palette of birds may be broader than previously appreciated.

What is the genetic basis of eggshell coloration? Substantial research on egg color in domesticated birds, especially chickens, has helped to clarify the details of protoporphyrin and biliverdin biosynthesis: both pigments are associated with heme, a critical compound for oxygen transport in the blood stream. Historically, numerous proteins and enzymes—including solute carriers, ATB-binding cassette transporters, and oxidases—have been described in the heme biosynthesis pathway (Bai et al. 2019), but the specific genes responsible for egg color remained elusive until 2 studies uncovered the basis of blue eggshell coloration in chickens. Wang et al. (2013) and Wragg et al. (2013) identified solute carrier *SLCO1B3* as an autosomal gene expressed during biliverdin deposition in the shell gland, controlled by an EAV-HP (retrovirus) insertion present only in blue egg-laying chicken breeds. Curiously, a different mechanism appears to be responsible for blue eggshell coloration in ducks. Capitalizing on the large degree of intraspecific eggshell color in mallards (*Anas platyrhynchos*) and mallard-derived domestic ducks, 2 recent studies have identified SNP variants in the *cis*-regulatory region of the autosomal *ABCG2* gene that causes the gene to be highly expressed in the shell gland (Chen et al. 2020; Liu et al. 2020). *ABCG2* is an ATP-binding cassette gene thought to transport biliverdin. Brown porphyrin eggshell color appears to be controlled by multiple genes, 7 of which—including *CPOX*, a heme biosynthesis enzyme that produces browner eggs when highly expressed—were identified in a chicken gene expression study (Zheng et al. 2014). The involvement of several of these genes (and corresponding proteins) in mediating brown eggshell color was confirmed by a sophisticated proteomics study that used iTRAQ (isobaric tags for relative and absolute quantitation) to quantify protein expression levels in the chicken shell gland (Li et al. 2016).

Despite these advances in domesticated fowl, very little is known about the genetic basis of eggshell color in the more than 10 000 species of wild birds. Are *SLCO1B3* or *ABCG2* genes responsible for the iconic blue eggshell color of American robins (*Turdus migratorius*) or the hundreds of other species that lay blue eggs? What genes are responsible for the intricate eggshell maculation (speckling) that appears in many avian lineages? When did pigmented eggshells originate—early in nonavian dinosaurs (Wiemann et al. 2018) or later in archosaurs (Shawkey and D'Alba 2019)? Which genes were involved in the pigmentation of early eggs? The next decade promises to be a pivotal one for eggshell coloration genetics. We wager that some of the most compelling breakthroughs will come from brood parasite–host systems in which parasites, like the common cuckoo (*Cuculus canorus*), are under extraordinary selection to evolve egg colors and patterns to match those of their target hosts (reviewed in Stoddard and Hauber 2017). In these systems, egg color and patterning are believed to be maternally inherited via the female-specific W sex chromosome (Gibbs et al. 2000). In common cuckoos, support for this idea comes from a recent population genetics study showing that the genomes of cuckoo females laying blue eggs (to match blue egg-laying hosts) differ from those of other cuckoo females only in their maternally inherited components (i.e., mitochondrial and

W-specific chromosome genes) (Fossøy et al. 2016). However, the specific genes responsible for blue egg color in cuckoos remain a mystery. Finally, although many birds lay blue-pigmented eggshells, blue pigments are absent from bird feathers, which are structurally colored. Why blue egg-laying birds have not evolved to deposit blue pigments in their feathers is an open question.

Functional and Evolutionary Perspectives on Avian Coloration: Insights From Genetics

In this final section, we describe 2 research areas that have been enlivened by recent breakthroughs in color genetics. These are: 1) genetic links between the visual system and carotenoid coloration, and 2) speciation.

Genetic Links Between the Visual System and Carotenoid Coloration

Diet-derived carotenoid pigments are common not just in bird feathers and bare parts but also in bird retinas, where they play an extremely important role in color vision. Birds have 4 types of cone photoreceptors that are maximally sensitive to violet or ultraviolet, blue, green, and red wavelengths of light (Hart and Hunt 2007); color vision is the result of post-retina comparison of the outputs from different cone types (Goldsmith 1990). The maximal sensitivities of the cones are roughly evenly spaced between 300 and 700 nm, which is predicted to optimize color discrimination. Additionally, 3 of the 4 cone types are paired with a carotenoid-containing oil droplet that acts as a long-pass filter, reducing the overlap between the spectral sensitivity curves of neighboring photoreceptor types and leading to enhanced color discrimination at the expense of absolute sensitivity (Bowmaker 1980; Vorobyev 2003; Toomey et al. 2016; Kelber 2019; Figure 3A,B). Cone oil droplets are widely distributed across vertebrates from fish to mammals (but not eutherian mammals). The presence and identity of pigments in oil droplets vary across taxa, but both turtles and birds are known to have cone oil droplets pigmented with carotenoids (reviewed in Toomey and Corbo 2017). The absorbance properties of the oil droplets—and thus their effects on color vision—are determined by the type and concentration of carotenoid present in each oil droplet.

Which came first in birds: carotenoids for oil droplets or for feathers and body parts? A deeper understanding of the genetic basis of carotenoid processing is helping to address this question. The oil droplet associated with the long-wave sensitive (LWS) cone in birds is pigmented with astaxanthin (Goldsmith et al. 1984; Figure 3B), a ketocarotenoid that is produced through modification of dietary yellow carotenoids by the carotenoid ketolase *CYPJ219*. As we discussed earlier, *CYPJ219* also performs this role in feathers and skin, converting yellow carotenoids to red ketocarotenoids in birds with red plumage. The presence of a *CYP2J19* ortholog in turtles (which possess oil droplets but typically lack red body coloration) and the presence of an intact *CYP2J19* gene in birds that have oil droplets but lack red ketocarotenoid body coloration support the idea that *CYP2J19* initially mediated synthesis of red ketocarotenoids in LWS cone oil droplets (Twyman et al. 2016, 2018a; Emerling 2018). *CYP2J19* tends to be pseudogenized or have reduced function in nocturnal birds (owls, kiwi) and aquatic foragers (penguins) (Emerling 2018), consistent with the hypothesis that oil droplets involve a tradeoff between light sensitivity and enhanced color discrimination. The pseudogenization of *CYP2J19* in species that lack red oil droplet filtering and coloration suggests that *CYP2J19* has no major role outside of color vision and coloration (Toomey and Corbo 2017; Emerling 2018). Thus, ketocarotenoid processing likely evolved in the context of the visual system before birds and turtles co-opted it for body color (Lopes et al. 2016; Twyman et al. 2016, 2018a). Moreover, co-option of *CYPJ219* from its ancestral function in the retina to a novel function in ketocarotenoid body coloration appears to be common in birds. Evolutionary transitions between yellow dietary carotenoid body coloration and red ketocarotenoid body coloration are widespread in birds (Olson and Owens 2005), with red body coloration evolving independently multiple times (Friedman et al. 2014; Twyman et al. 2016).

Although *CYP2J19* copy number variation has so far been examined in only a small fraction of bird species, most species have a single copy (Mundy et al. 2016; Emerling 2018; Twyman et al. 2018a). These results suggest pleiotropy of *CYP2J19* in the visual system and carotenoid-based ornamentation, so that a single gene controls ketocarotenoid processing in oil droplets and in red plumage. The only documented exception is the zebra finch (*T. guttata*), which possesses 2 *CYP2J19* copies: one expressed only in the retina and the other expressed mainly in the beak and tarsus (Mundy et al. 2016). In general, pleiotropy of *CYP2J19* might constrain the evolution of ketocarotenoid coloration because changes to *CYPJ19* that would disrupt its function in the visual system would be selected against. Duplication and subfunctionalization of *CYP2J19* might then enable innovations in carotenoid coloration in the integument. However, whether *CYP2J19* duplication and subfunctionalization have resulted in innovations in zebra finch coloration is not clear (Mundy et al. 2016). Assessing copy number variation or other signatures of selection on *CYP2J19* in relation to visual ecology and carotenoid coloration across broader swaths of avian diversity would likely be a fruitful area of research; the recent publication of 363 avian genomes (Feng et al. 2020) provides an opportunity to explore more comprehensively how the genetic links between *CYP2J19*-mediated carotenoid coloration in the retina and in integumentary coloration constrain or facilitate the evolution of carotenoid coloration.

In addition to *CYP2J19*, 2 other genes are known to affect carotenoid pigmentation in both oil droplet and integument (feather and skin) coloration: *BCO2* and *SCARB1* (see "The Genetics of Pigmentary Plumage Colors: Carotenoids and Psittacofulvins" section) (Figure 3B). *BCO2* is responsible for the first step in an enzymatic pathway that converts dietary carotenoids to the apocarotenoids that pigment the SWS2 oil droplet (Toomey et al. 2016), and *SCARB1* mediates carotenoid uptake into the retina (Toomey et al. 2017). Mutations in both *BCO2* and *SCARB1* pleiotropically alter the accumulation of carotenoids in body coloration and the retina (Figure 2B,C) (Toomey et al. 2017; Gazda et al. 2020b). The importance of these links to the evolution of coloration is not as clear as in the case of *CYP2J19*, however, because both genes have significant functions outside of coloration and color vision—*BCO2* in carotenoid homeostasis (reviewed in von Lintig 2010; Lobo et al. 2012) and *SCARB1* in lipid transport in a variety of contexts (Williams et al. 2000; Kiefer et al. 2002; Connelly and Williams 2004; van Bennekum et al. 2005). Both genes are evolutionarily ancient and have conserved functions across broad taxonomic scales (Kusakabe et al. 2009; Holmes and Cox 2012; Toews et al. 2017). How did these genes come to be involved in color vision and coloration in birds, and how does selection from multiple sources shape their current function?

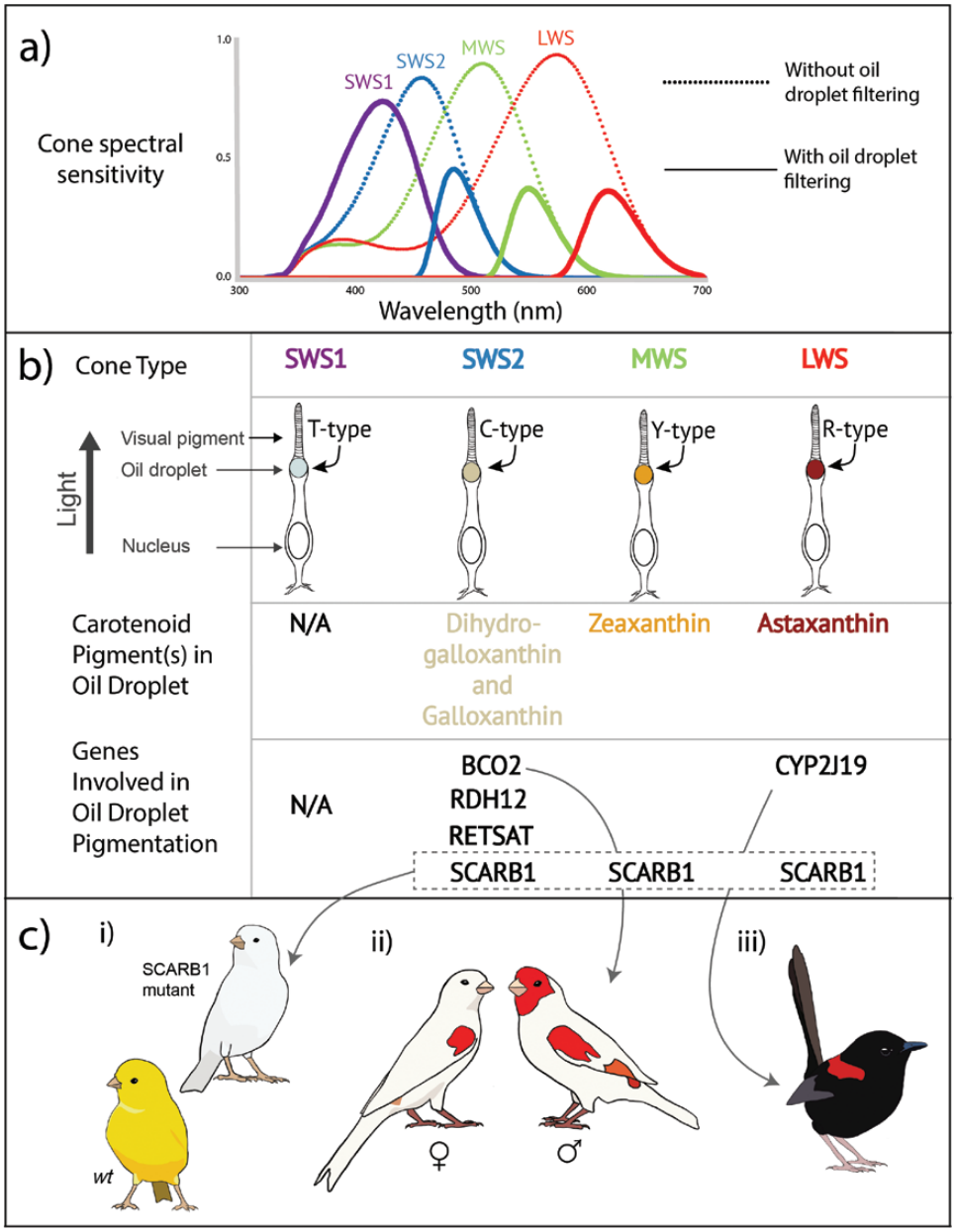


Figure 3. Shared mechanisms of carotenoid processing in the visual system and integument. (**A**) Spectral sensitivities of 4 avian cone types. Oil droplet filtering narrows the width of spectral sensitivity curves and reduces overlap between curves for different photoreceptor types. Data shown are for a violet-sensitive species. (**B**) The 4 avian cone types, associated oil droplets and their pigments, and known genes involved in carotenoid pigmentation of the oil droplets. (**C**) Examples of systems where the same genes involved in the visual system are involved in integument pigmentation. (i) A mutation in *SCARB1* results in depigmentation of carotenoids from feathers and retina in common canaries (*Serinus canaria*; Toomey et al. 2017). (ii) *BCO2* upregulation in female *mosaic* canaries (*Spinus cucullatus* × *Se. canaria*) results in sexual dichromatism of carotenoid-based plumage Gazda et al. (2020a). (iii) Testosterone regulation of *CYP2J19* results in redder feathers in the red-backed fairy wren (*Malarus melanocephalus*; Khalil et al. 2020). (**A**) Reproduced with permission from Toomey et al. (2016).

Because carotenoid expression in birds represents an intriguing link between the production and perception of colorful signals, further exploration of carotenoid genetics is likely to be revealing. Across species, how common are duplications and losses in these genes? What were the ancestral functions of these genes, and how have they been co-opted for new functions? Across *and* within species, how does variation in carotenoid pigmentation in the oil droplets affect perception of visual signals? The answers to these questions are well within reach. Experimental (Toomey and McGraw 2012; Caves et al. 2020) and theoretical (Lind et al. 2017; Ronald et al. 2017) studies on the effects of oil droplets on perception are proliferating—and so far suggest that interactions among carotenoid content, color discrimination, and visual signals are complex. The emergence of new techniques—particularly single-cell transcriptomics, which was just used in a detailed study of the chicken retina (Yamagata et al. 2021)—should galvanize efforts to clarify the genetic basis of carotenoid expression and evolution in birds.

Speciation

Identifying the genetic basis of color traits has opened up new areas of inquiry into speciation. Plumage color and patterns are often important for species recognition and mate choice. Many closely related species differ in plumage patterning or other plumage traits, suggesting that divergence in plumage may be linked to speciation in some groups (West-Eberhard 1983; Price 1998; Edwards et al. 2005; Seddon et al. 2013; Gomes et al. 2016; Price-Waldman et al. 2020), but the genetic mechanisms for differentiation have been difficult to pinpoint. Recent genome-scale analyses have shown that the genomes of some closely related, recently diverged species differ primarily in genes related to pigmentation, including *Sporophila* seedeaters (Campagna et al. 2017), carrion and hooded crows (Poelstra et al. 2015; Knief et al. 2019), *Vermivora* warblers (Toews et al. 2016b), *Lonchura* munias (Stryjewski and Sorenson 2017), and *Setophaga* warblers (Brelsford et al. 2017; Baiz et al. 2020b; Wang et al. 2020). These results suggest that prezygotic isolation may arise through the evolution of relatively few loci of large effect that control the production of colorful traits that are important for species recognition or mate choice (Funk and Taylor 2019). Across 36 warblers in the genus *Setophaga*, 2 peaks of divergence include *ASIP* and *BCO2* (Baiz et al. 2020b). A gene tree of *ASIP* is largely concordant with the species tree for the *Setophaga* radiation, suggesting that repeated, independent mutations in *ASIP* have contributed to differences in melanin-based plumage across warblers. In contrast, variation in *BCO2* is notably discordant from the species tree, suggesting that introgression of *BCO2* has contributed to changes in carotenoid-based plumage across *Setophaga* warblers. While specific mutations in *BCO2* identified in warblers have yet to be functionally validated as altering the carotenoid breakdown and the expression of carotenoid traits, these patterns of *BCO2* introgression represent the clearest evidence so far of functional gene transfer of carotenoids in vertebrates (Baiz et al. 2020b) and provide parallels to the adaptive introgression of melanin genes in mammals (Caro and Mallarino 2020). In 2 of these warblers, *Setophaga townsendi* and *Setophaga occidentalis*, multiple plumage regions (cheek, crown, breast, and flank) are controlled by a single gene block (ASIP-RALY) (Wang et al. 2020). Selection on the ASIP-RALY gene block maintains a stable hybrid zone between the 2 warblers (Wang et al. 2020), likely as the result of opposing dominance of alleles that results in signal breakdown in heterozygotes (de Zwaan et al. 2021). Other recent hybrid zone studies have revealed that unusual patterns of introgression can be explained by epistasis and dominance in the loci underlying plumage traits (Knief et al. 2019; Semenov et al. 2021). Overall, identifying the genetic bases of plumage traits that differ across species radiations can help identify how hybridization and parallel evolution in coloration genes may be linked to species divergence.

Concluding Remarks

Understanding the genetic bases of coloration is key to understanding the origins and evolution of phenotypic variation (Hubbard et al. 2010; Orteu and Jiggins 2020). Until recently, the molecular and genetic mechanisms underlying some of the most colorful avian traits were completely unknown. Fortunately, substantial gains have been made in identifying the genetic and developmental bases of diverse bird coloration. Colors in birds are produced by a wide range of pigmentary and structural mechanisms in their feathers, skin, feet, bills, irises, and eggs. Recent progress on the genetics of carotenoids, psittacofulvins, and structural colors has expanded the field beyond the better-studied melanin pigments, with an increasing number of studies on coloration in wild birds and on bare parts and eggs (summarized in Box 1). Although much of avian color diversity remains enigmatic from a genetic standpoint (Figure 1, Box 3), this recent progress is promising. We believe that further breakthroughs are imminent and will continue to offer new insights into avian biology and evolution.

Finally, we note that recent advances in coloration genetics have been complemented by advances in the genetics of the visual system. Because the perception of colors involves visual systems, integrating the genetics of coloration with the genetics of the visual system has been a longstanding goal of evolutionary biology (Hubbard et al. 2010). Is variation in color linked to variation in color perception, and do the genes underlying changes in coloration coevolve with the genes related to visual perception? This is an intriguing but unresolved question in many colorful groups of animals, with clearest evidence for links between variation in color and vision coming from butterflies and fishes (reviewed in Osorio and Vorobyev 2008; Price 2017; Cummings and Endler 2018). Above, we described recent work on the genetics of carotenoid-based oil droplets in the retina and their links to carotenoid coloration in the integument. In addition to these discoveries, extensive work on the genetics of opsin pigments—the photosensitive pigments in the cone photoreceptors—has helped to unravel the molecular basis and evolutionary history of color vision in birds (Hart 2001; Hart and Hunt 2007; Bloch 2016; Kelber 2019). The application of retinal transcriptomics and genomics has revealed conservation of opsins in some groups (Coyle et al. 2012; Casalía et al. 2021) but a surprising amount of variation in opsins in others, including loss, pseudogenization, variation in expression levels, or adaptive molecular evolution of some opsins (Ödeen et al. 2012; Knott et al. 2013; Ödeen and Håstad 2013; Bloch 2015; Borges et al. 2015; Fidler et al. 2016; Wu et al. 2016; Höglund et al. 2019; Feng et al. 2020). Whether avian color diversity is shaped by variation in sensory systems is an open question, but recent advances in the genetics of coloration and color vision make addressing this question increasingly possible. Future studies in avian coloration genetics should continue to investigate

Box 3 Outstanding Mysteries and Future Questions

- Are red and yellow psittacofulvins differentiated by their chemical structures, interactions with feather keratin, or both?
- How are changes in coding regions versus regulatory regions associated with changes in coloration, especially for different color production mechanisms?
- What are the genetic bases of structurally colored feathers and skin? How do genetically encoded factors set the stage for self-assembly in the development of these colors?
- How are different color production pathways co-expressed, co-regulated, or otherwise associated to create the diverse colors we see across birds?
- Why are some coloration mechanisms taxonomically and anatomically restricted? Why are blue pigments found only in eggshells? Why do parrots deposit red and yellow carotenoids in bare parts but red and yellow psittacofulvins in feathers? Why do bare parts generally lack patterning?
- Which evolved first: genes involved in visual perception (e.g., opsins, genes involved in oil droplets) or genes involved in coloration? Do visual perception genes coevolve with coloration genes?

the molecular and genetic bases of diverse mechanisms that color the skin, feathers, eyes, and eggs of birds. In particular, efforts to understand how different color production pathways interact and how the visual system influences color diversity (Box 3) are likely to be productive.

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Data Availability

No data are associated with this article.

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