CHAPTER 1. CHARACTERIZING AND QUANTIFYING COLORATION

Dakota E. McCoy[1,2]

[1] Department of Ecology and Evolution, The University of Chicago, Chicago, IL, USA

[2] Marine Biological Laboratory, Woods Hole, MA, USA

1.1 What is color?

Color is tricky. Color depends on two exceedingly complicated things: *light* and *sensory systems* (as well as the comparatively simple reflecting/absorbing object or surface). The first, light, has baffled humans for generations. We can predict how light behaves, but we struggle to intuitively describe it. Light behaves as both a particle and a wave. In fact, all particles behave as both a particle and a wave– it is just especially noticeable with light! Consider the bizarre double-slit experiments. When scientists send a beam of light through two parallel slits, an interference pattern of light and dark bands appears on the screen behind (a wave-like phenomenon), even if you send the light through one photon at a time. But if you detect which slit each photon goes through, then each photon behaves like a particle and the interference pattern disappears—the observer effect. Long story short, no one has described light in a way that intuitively makes sense, and it's "unlikely that anyone ever will" (Johnsen 2012).

Luckily, to study bird color, we need not discuss of what light *is*. For our purposes, light is electromagnetic radiation that varies in wavelength. What we consider to be visible light is a small slice of the electromagnetic spectrum that also includes gamma rays (tiny wavelengths < 10^{-11}m), x-rays (small wavelengths ~0.01-10nm), ultraviolet radiation (~10-400nm), infrared radiation (larger wavelengths ~700nm-1mm), and radio waves (huge wavelengths ~1mm-100km). The wavelengths that matter to biologists who study color, for reasons you'll learn below, range roughly from 300-700nm (plus or minus 20nm at either end). We call this the UV-visible spectrum, even though it leaves out the subset of UV radiation <300nm. Birds flaunt beautiful colors across this entire rainbow (Figure 1.1).

<<Figure 1.1 about here>>

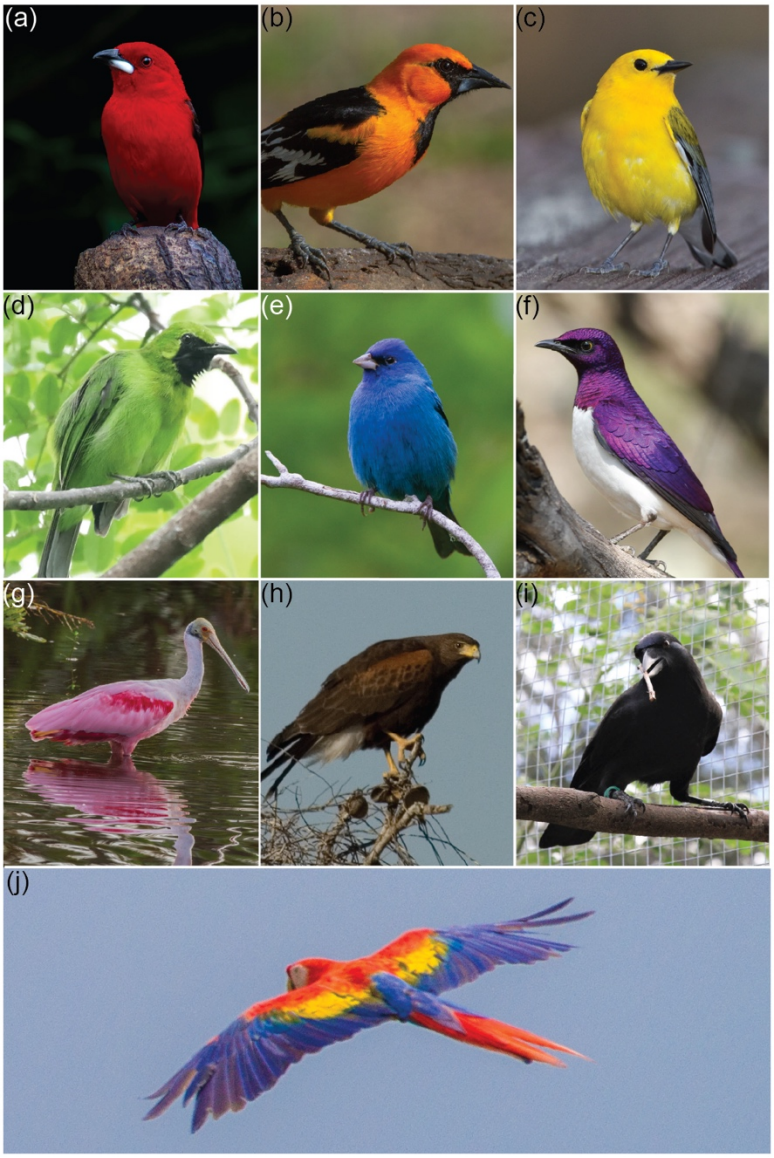

Figure 1.1. Birds flaunt colors across the entire rainbow, from red to violet (and many combinations thereof). **(a)** Brazilian Tanager *Ramphocelus bresilius*. **(b)** Altamira Oriole *Icterus gularis*. **(c)** Prothonotary Warbler *Protonotaria citrea*. **(d)** Lesser Green Leafbird *Chloropsis cyanopogon*. **(e)** Indigo Bunting *Passerina cyanea*. **(f)** Violet-Backed Starling *Cinnyricinclus leucogaster*. **(g)** Roseate Spoonbill *Platalea ajaja*. **(h)** Harris's Hawk *Parabuteo unicinctus*. **(i)** New Caledonian Crow *Corvus moneduloides*. **(j)** Scarlet Macaw *Ara macao*. Photo credits: (a) Enéas V. Gouvêa, (CC BY-SA 4.0, https://creativecommons.org/licenses/by-sa/4.0/). (b-c, e, g-h, j) Geoff Hill. (d) Tony Castro, (CC BY-SA 4.0, https://creativecommons.org/licenses/by-sa/4.0/). (f) Derek Keats (CC BY 2.0 Generic https://creativecommons.org/licenses/by/2.0/deed.en). (i) Dakota McCoy.

That brings me to the second complicated thing that color depends on: sensory systems. Humans do not see color in the same way that birds do, or their predators, or their prey. We may well see

four different red birds and label them all "red," only to learn that four totally different physical properties have produced four visual signals that birds likely perceive to be distinct (Figure 1.2). Such colors that look the same, but have different compositions and spectral properties, are "metamers". We cannot see ultraviolet (UV) light, and many birds can – among other sensory differences (see Chapter 2 for a full overview of the fantastic sensory systems of birds.) For these reasons, biologists clamor for objective means to assess color. However, it is worth noting that human-perceived bird color captures most of the variation in visible bird color, correctly clustering species, even though we fall short on objective color (Bergeron and Fuller 2018; see Eaton 2005). Your eyes can and should be a good starting point for research ideas about bird color. Many students come up with research questions after spotting a strange bird in the wild or on a museum shelf!

Color should be quantified through objective measures that do not depend on the human visual system (Endler 1990). Color is best measured "with a *spectro*-something" (Andersson and Prager 2006). Indeed, most commonly, ornithologists measure color with a **spectrophotometer** (alternatively called a **spectroradiometer** or **spectrometer**; ornithologists most often use the term spectrophotometer, so that is the term I use herein). These devices collect light at each wavelength that reflects from a bird's plumage or bare parts (the bird's "integuement"); often, these instruments also illuminate the integument with known wavelengths of light. Spectrophotometers produce a **reflectance curve**. A reflectance curve plots the wavelengths of light on the x-axis versus the percent of each wavelength of light that is reflected on the y-axis (Figure 1.4). Wavelengths correspond to the sensory phenomenon of color, such that, e.g., what we think of as green light has ~ 495-570nm wavelength. A green bird reflects a lot of green light (495-570nm) and little light of other colors. This reflectance curve will be our workhorse– our objective color measurement from which further analyses proceed.

In this chapter, I will describe the possible fates of a photon when it interacts with biological tissue (Section 1.2); explain general principles of measuring reflectance (1.3); give an overview of measurement techniques like spectrophotometry (1.4); describe how we can extract metrics like hue from those measurements (1.5), discuss a useful plot type called the tetrahedral colorspace (1.6), and summarize how optical modeling can complement experimental investigations of color (1.7). Throughout, I place particular emphasis on post-2006 findings, developments, and opportunities in avian color quantification. In the past 20 years, ornithologists have benefited from (i) open-source code packages that analyze large amounts of color data; (ii) affordable, durable tools for reflectance spectrophotometry; (iii) novel, still-developing methods of hyperspectral imaging, which may be the future of color quantification; and (iv) user-friendly optical modeling software. Further, I predict major opportunities to study (iv) gloss and related phenomena; (v) colors in bare parts, eyes, and other non-plumage regions, particularly in non-passerine birds; and (vi) to what extent birds produce and react to signals that incorporate fluorescence (likely), polarization (possible), and upconversion (unlikely). Together, I hope that

this chapter will help students chart a course forward to probe a beautiful feature of our natural world: the brilliant colors of birds.

<<Figure 1.2 about here>>

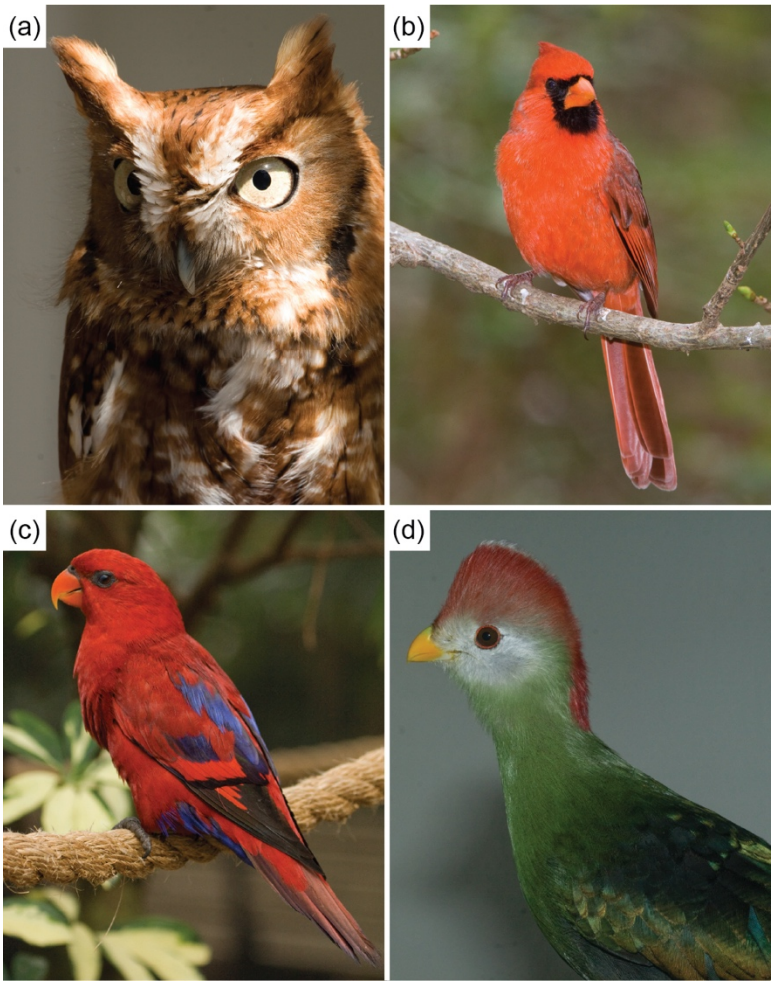

Figure 1.2. Four species of bird have red plumage patches that are all produced by different physical mechanisms. **(a)** The Eastern Screech Owl *Megascops asio* has reddish-brown plumage colored predominantly by pheomelanin pigment, **(b)** Northern Cardinal *Cardinalis cardinalis* gains its red color from carotenoid pigments, **(c)** Red Lory *Eos bornea* appears bright red due to parrot-specific pigments called psittacofulvins, and **(d)** Red-crested Turaco *Tauraco erythrolophus* has a bright red crest due to turaco-specific turacin pigments. Photos by Geoff Hill.

1.2 The fates of a photon

1.2.1 Light can be absorbed or scattered by birds to produce color

Birds do not create light. Some animals do, from bioluminescent fish in the deep sea to fireflies in a forest. In these cases, scientists analyze chemical reactions that produce photons. Scientists studying bird coloration need only consider the how surfaces of birds absorb or scatter sunlight to produce color (fluorescence is a special case, discussed below). In the following paragraphs I provide a brief overview of how birds produce color, strongly inspired by and based on Sönke Johnsen's (2012) book *The Optics of Life.*

When light encounters a bird, or indeed any biological tissue, it can meet one of two fates. A photon can be **absorbed** or **scattered.** Color cannot be generated by absorption alone; scattering is required, too (Johnsen 2012). **Reflectance** and **transmittance** are special cases of scattering. Often, biologists measure how much light of each wavelength is reflected, assuming that all other light is absorbed or transmitted.

If a photon is **absorbed**, its energy is transferred to an atom or molecule, usually causing an electron to jump to an excited state. The difference in energy between the low-energy orbital state of the electron and the high-energy orbital state must equal the energy level of that photon. By this process, pigments produce coloration. Pigments absorb certain wavelengths of light, but not others. Confusingly, we typically name pigments based on the colors of light that they *do not* absorb. Chlorophyll absorbs strongly in the blue and red wavelengths, but we consider it to be a "green" pigment (which describes the reflected, perceived light). Carotenoids absorb strongly in the blue-green portion of the visible spectrum, but we think of them as yellow-orange-red pigments (Figure 1.2b).

If a photon is **scattered**, the light wave propagates in some direction. Scattering can be elastic, where the emitted photons match the color (and energy) of incident photons, or inelastic, where the photons "out" do not match the photons "in". Inelastic scattering is largely irrelevant to bird coloration, except that it is useful as a tool to identify certain molecules through Raman spectroscopy (e.g., by which scientists discovered a new yellow pigment in penguins (Thomas et al. 2013)). When we speak of a red bird, we are talking about a bird that **elastically scatters** (reflects) red light wavelengths and absorbs remaining UV and visible light wavelengths. Structural color in birds arises from small physical structures that interfere with and scatter light, often in combination with absorbing pigments (see (Prum 2006) and Chapter 7 herein).

Reflectance is often described as being either **specular** or **diffuse**. Specular reflectance, also known as gloss, occurs when light interacts with a smooth surface and reflects parallel rays at a consistent angle: the angle of incidence equals the angle of reflectance. Specular reflectance is mirror-like. In contrast, diffuse reflectance arises from rougher surfaces, from which incident light is scattered over many angles. Diffuse reflectance can also be called matte, especially if you are in the paint aisle of a hardware store. A male House Sparrow's black bib is matte (*Passer*

domesticus), while a Common Raven's plumage is glossy (*Corvus corax*). Many recent studies have revolutionized our understanding of gloss—both its physical basis and how best to measure it (Eliason and Clarke 2020; Iskandar et al. 2016; Maia et al. 2011; Toomey et al. 2010); see section 1.5.2).

Absorption and scattering both depend on fundamental material properties of the tissue. For example, the refractive index of a material determines how fast light moves through it; most biological substances have a higher refractive index than air, and so they substantially bend and scatter light. Air's refractive index is set as n_{air}=1, while key components of bird feathers—e.g., keratin and melanin, have higher refractive indices around 1.55 and 1.75. Melanin in particular has a high refractive index among biomaterials. Note that refractive index actually varies with wavelength and includes an imaginary component that describes light absorption and attenuation. Leertouwer et al. (2011) carefully measured the real refractive index of keratin $n_{keratin}$ in White Goose *Anas anas* feathers at three wavelengths using Jamin-Lebedeff interference microscopy: $n_{keratin}$ = 1.572 for 400nm light, =1.552 for 500nm, and =1.541 for 600nm—fitting the Cauchy equation: $n_{keratin}(\lambda) = 1.532 + \frac{5.89 \times 10^3 nm^2}{\lambda^2}$. Here, λ is the Greek letter lambda, which is shorthand for wavelength. Using similar methods on the Bird-of-Paradise *Parotia lawesii*, Stavenga et al. (2015) report that the real refractive index of melanin in barbules varies from $n_{melanin}$ = 1.8-1.7 over the visible light range 400-700nm (Stavenga et al. 2015).

1.2.2 Fluorescence and upconversion change the color of light

Fluorescence is a special case of absorption. Fluorescence is not glowing; fluorescence does not make light (Johnsen 2012). Certain pigments are the workhorses that fluoresce. Fluorescent pigments absorb the energy of a photon, and then emit only part of that energy back out as a lower-energy, longer-wavelength photon. What happens to that extra energy that is not emitted as a photon? It is released as vibration or heat. Scientists characterize fluorescence with two curves. The **excitation curve** shows what wavelengths excite the molecules and can be thought of as the probability that a photon of each wavelength is absorbed. The **emission curve** or **emission spectrum** plots the wavelengths of the emitted photons (Figure 1.3).

Fluorescence seems to be a by-product in many biological tissues, a side effect of molecular structure rather than a trait subject to selection. But certain birds likely use fluorescence as sexual or social signals. In Budgerigars (*Melopsittacus undulatus*), yellow feathers absorb UV light and emit yellow. Both females and males strongly prefer plumage with its natural fluorescence (rather than experimentally manipulated to have lower fluorescence; Arnold et al. 2002). Recently, scientists used spectrophotometry with blue excitation light (440−460 nm) to show that, in all core species of Birds-of-Paradise, males fluoresce in their colorful plumages, mouths, bills, and even feet (Figure 1.3, (Martin et al. 2025)). Martin et al. (2025) also confirmed that UV light excited fluorescence in the Birds-of-Paradise, and they measured using blue light for safety

reasons. It remains to be seen how perceptually significant the Bird-of-Paradise fluorescence is; see Martin et al. (2025) for a convincing argument that fluorescence likely is perceivable and used in sexual displays. Rhinoceros Auklets *Cerorhinca monocerata* (Wilkinson et al. 2019), Crested Auklets *Aethia cristatella* (Wails et al. 2017), and Atlantic Puffins *Fratercula arctica* (Dunning et al. 2018) have fluorescent ornaments in their bills. Current students have an opportunity to assess other bird clades for fluorescence, and pair these spectrophotometric investigations with sensory or behavioral experiments to determine whether any fluorescent ornaments serve a purpose.

A similar process, so far unknown in nature, is called **upconversion.** Through upconversion, two or more photons of higher wavelengths are combined to emit a photon of lower wavelength (this high-to-low wavelength shift is opposite to low-to-high fluorescence). Scientists design upconverting nanoparticles for medical imaging, to sense processes and forces inside a living body (see (Casar et al. 2025; Lay et al. 2017)). Will we one day discover an upconverting bird, butterfly, or microalgae? It seems unlikely, but never say never.

<<Figure 1.3 about here>>

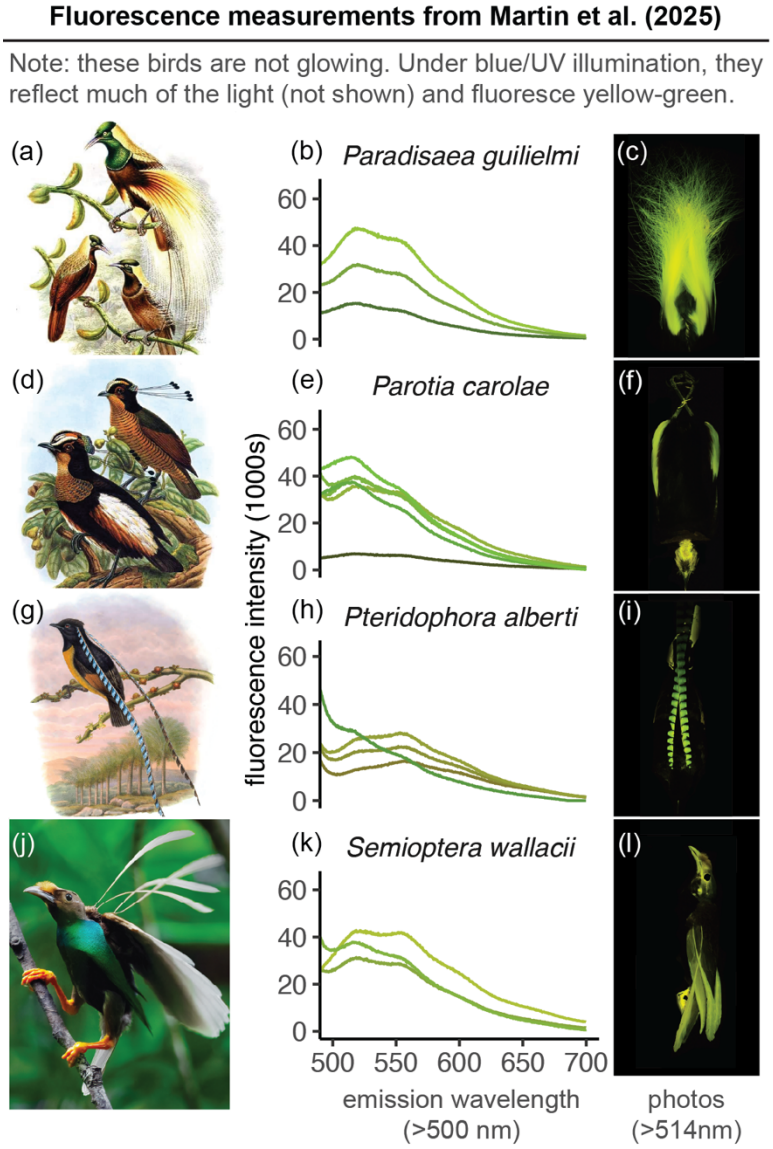

Figure 1.3: Martin et al. (2025) report yellow-green fluorescence in birds-of-paradise under blue illumination. Note that these birds are not glowing, nor are they glowing in the dark; rather, when illuminated with blue or UV light, they reflect lots of the blue and UV light (not shown here) and also fluoresce some of the light, emitting yellow-green photons. The reflectance spectra and photographs here exclude reflected blue and UV light. Species include the **(a-c)** Emperor Bird-of-Paradise *Paradisaea guilielmi* **(d-f)** Carola's Parotia *Parotia carolae* **(g-i)** King of Saxony Bird-of-Paradise *Pteridophora alberti* and **(j-l)** Standardwing Bird-of-Paradise *Semioptera wallacii.* Middle column **(b,e,h,k)** show the emission spectra for plumages excited by blue light (wavelength 420-460 nm); note that the X-axis is cropped at 500 nm to exclude the substantial reflected blue light. Right column **(c, f, i, l)** shows photographs taken under blue excitation (420-460 nm) with a longpass filter that excludes light of wavelengths <514 nm (including the substantial reflected blue or UV excitation light). To color reflectance curves by

approximate human-perceived color, I used the R package pavo (Maia et al. 2019); to plot and wrangle data, I used dplyr, stringr, tidyr, and ggplot2 (Wickham 2015; 2019; Wickham et al. 2020; Wickham 2016). Illustration and photo credits: (a, d, g) Bowdler Sharpe, public domain; (j) JJ Harrison (CC BY-SA 4.0, https://creativecommons.org/licenses/by-sa/4.0/).

1.3 We measure reflectance to quantify color: general principles

To quantify and analyze color, color researchers use one of several methods to measure reflectance (see following sections). That is, the scientist shines light of known wavelengths on a bird's integument and then measures the percentage of light at each wavelength that reflects back. As a result, a reflectance curve can be plotted (Figure 1.4d-f): on the x-axis is the wavelength of light, from ultraviolet (300nm) through violet, blue, green, yellow, orange, and red (ending at 700 nm). For the red-orange ventral plumage of the golden-bellied starfrontlet *Coeligena bonapartei*, the reflectance curve peaks around 620nm, squarely between what humans call orange (~590-620nm) and red (~620-700nm (Figure 1.4)). It reflects the most light in that red-orange region, and under standard lighting those red-orange photons interact with our eyes and brains to produce an orange color perception; other colors of light are absorbed by the feathers or scattered away from our eyes (low reflectance elsewhere in the spectra, from 300-500 nm). Before I describe the specific methods of measuring reflectance (Section 1.4), I will present a brief overview of general considerations when measuring reflectance.

<<Figure 1.4 about here>>

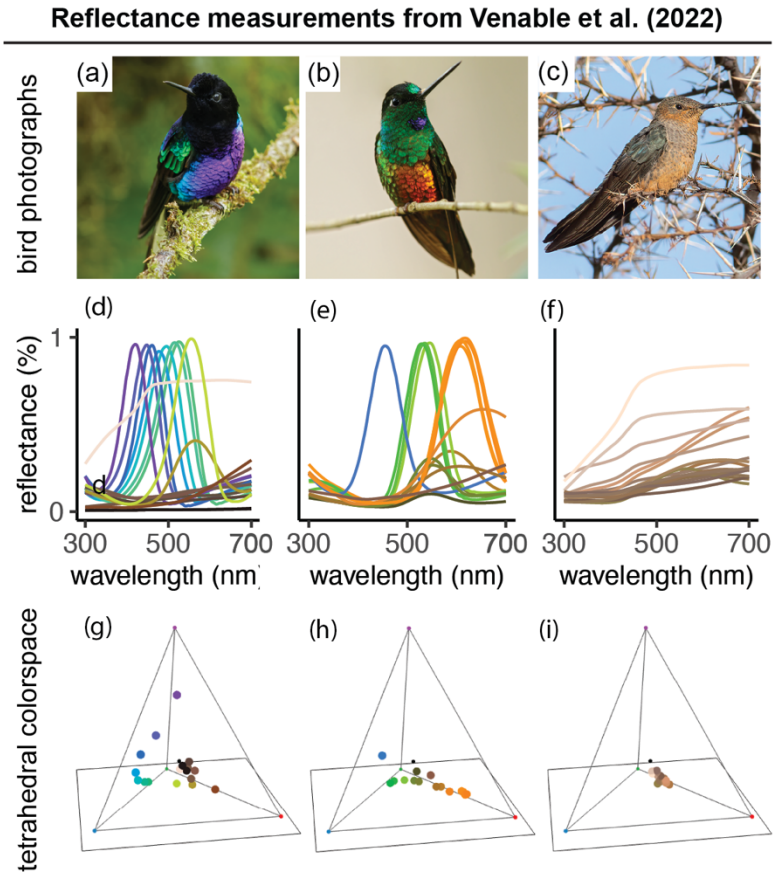

Figure 1.4. Hummingbirds exceed the known color gamut of all birds (Venable et al. 2022), making them the perfect case study to illustrate (d-f) reflectance spectra and (g-i) tetrahedral color space plotting (see section 1.6 for overview of tetrahedral colorspace). Photographs show **(a)** the Velvet-Purple Coronet *Boissonneaua jardini*, **(b)** the Golden-Bellied Starfrontlet *Coeligena bonapartei*, and **(c)** the Patagonian Giant Hummingbird *Patagonia gigas* (see new research that discovered two cryptic species of giant hummingbird; Williamson et al. 2024). Here, I replot data from Venable et al. (2022) to illustrate the many reflectance peaks from **(d)** violet to lime green in *Boissonneaua jardini;* **(e)** blue, green and orange in *Coeligena bonapartei*, and **(f)** the comparatively dull browns of *Patagonia gigas*. In a tetrahedral colorspace (see section 1.6), the color gamut varies from **(g)** the most colorful hummingbird *Boissonneaua jardini*, which occupies 13.8% of the VS color space, to **(h)** the golden-bellied starfrontlet *Coeligena bonapartei* at 4.28%, to **(i)** the least colorful measured hummingbird *Patagonia gigas* at 0.0725%. Reflectance curves are colored according to an estimate of how humans perceive the color each curve represents. Photo credits: (a-b) Glenn Bartley, (c) Daniel Field. I plotted the tetrahedral colorspaces using R package pavo (Maia et al. 2019) and wrangled data using stringr, tidyr, dplyr, and ggplot2 (Wickham 2015; 2019; Wickham et al. 2020; Wickham 2016).

1.3.1 White and dark reflectance standards set our endpoints

To objectively quantify color, we measure reflectance curves, which plot the proportion of light energy reflected at each wavelength of light. Reflectance varies from 0%-100%, where 100% is set by a **reflectance standard** – a vividly white material that diffusely reflects ~100% of incident photons across an entire 180° field of view (Figure 1.6). Common white standards include Spectralon(R) (Ocean Optics), PTFE(R) (Avantes), barium sulfate, magnesium oxide, or even Teflon(R) tape (Andersson and Prager 2006). The 0% dark standard can be achieved by blocking light from entering the measurement system (McCoy et al. 2018) or by purchasing a reflectance standard with known, low reflectance (e.g. 2% reflectance standard from Avantes; Dunning et al. 2023). Generally, the choice of reflectance standard is not overly important, because we typically compare within a population or across species or birds, which have roughly similar morphologies. It is very important to keep white standards clean or use a fresh standard with each measurement session; otherwise, the white standard may slowly get dirty and darken over several months, confounding your entire set of measurements.

Reflectance standards are very different from bird feathers. Most importantly, most reflectance standards diffusely reflect light with even scattering over many angles. Feathers often have a large component of glossy reflectance and a non-uniform component of diffuse reflectance. Therefore, reflectance standards may lead us to overestimate a feather's "true" brightness. Also, reflectance standards are flat but bird integuments are not. When performing spectrophotometry, it is important to use the same geometry for the plumage measurement and the reflectance

standard. This requires placing the reflectance standard in the exact same orientation, location, and distance to the light source and probe as for the feather(s) or integumentary surface.

Further, birds are fantastic photonic engineers that can challenge the limits of engineered white and black standards. Birds have invented super black feathers that reflect <0.01% of light (e.g. Bird-of-Paradise Princess Stephanie's astrapia *Astrapia stephaniae*; McCoy et al. 2018) and ultra white feathers than reflect >~50% of light (e.g. the Eurasian Woodcock *Scolopax rusticola*; Dunning et al. 2023). The woodcock's feather ramus alone reflects more than 100% of light compared to a diffuse white standard (Dunning et al. 2023). Even with such extreme reflectance profiles, researchers can proceed with best practices. Pick a reasonable standard with known reflectance, note the exact model, and note whether the reflectance standard is diffuse or glossy.

1.3.2 Light reflects over all possible angles of a hemisphere, but we usually measure just one angle

When light reflects off of a bird's integument, photons can travel in any direction. Sometimes plumage reflects light in a mirror-like manner, creating a smooth glossy surface. Sometimes, different colors of light reflect at different angles, causing iridescent plumage. Sometimes, color is selectively reflected toward a female or away from an interested predator. However, most often scientists collect reflected light only at one angle. This is a drawback of nearly all studies of color in birds! See Section 1.5.2 on Iridescence and Gloss for a description of angle-dependent spectrophotometry to address this drawback.

1.3.3 It is best to measure whole color regions rather than smaller parts.

Some studies pluck individual bird feathers, or tufts of feathers, while others directly measure a living bird or a museum skin. All of these approaches are valid, useful, and shaped by real constraints. However, it is preferable to measure color directly on a living bird or preserved museum skin (with intact plumage) for several reasons.

First, if you pluck one or several feathers, the background conditions strongly influence reflectance curves. For example, whether you measure a blue feather against a white or black notecard dramatically changes the result (Hubbard and Williard 2023).

Second, scientists have shown that plumages are multilayered, bird skin is itself colorful, and these layers have strong effects on the overall plumage color. For example, colorful tanagers (*Tangara* spp.) enhance their rainbow of colors with hidden layers of black and white feathers (Price-Waldman et al. 2025). These achromatic color layers determine how much backscattered light interacts with pigmented (and structurally colored) feathers, strongly changing the color. Further, bird skin itself manipulates light in a manner that influences perceived color – in bare skin (Prum and Torres 2003; Justyn et al. 2023) and also skin beneath plumage (Nicolaï et al. 2020). To accurately capture the real color signal perceived by birds, single plucked, or singly

measured, feathers would not capture the effect of feather layers and colored skin. Note that, even with museum specimens, color typically preserves more poorly in soft tissue like skin compared to features – an inherent limitation of museum work (Burns et al. 2017).

Third, the specific arrangement of feathers – their orientation, density, and degree of overlap – influences color. Many fantastic studies demonstrate this; see Section 1.5.2 on Gloss and Iridescence. Structures that produce gloss (Maia et al. 2011; Iskandar et al. 2016), iridescence (Giraldo et al. 2021; Stavenga et al. 2011), and even super black (McCoy et al. 2018; McCoy and Prum 2019) are angle-dependent and may behave differently if plucked and flattened on a cardstock substrate. That said, many studies of iridescence intentionally pluck and flatten feathers to make analysis simpler (by removing any bias from the curve of the bird's body; see Iskandar et al. 2016).

All this to say – if you can measure feather reflectance directly on the bird, do so. If you cannot, keep in mind possible confounds: layered plumage, colored skin, what background substrate you choose, and the orientation and arrangement of feathers. In some cases, you may wish to isolate individual regions of a feather for careful reflectance measurements – e.g. to determine how the barb vs. the ramus contributes to color using microspectrophotometry.

1.3.3 Challenges and opportunities in quantifying color of eyes, bills, feet, and other bare parts

Plumage receives lots of attention, but birds also have brilliantly colored eyes (Figure 1.5), eggs, and bare parts such as bills and feet. A major frontier of bird color science lies in quantifying and analyzing bare part coloration (Price-Waldman and Stoddard 2021; Iverson and Karubian 2017). With bare parts, scientists have an opportunity to use photography (Section 1.4.2), multi- or hyperspectral imaging (1.4.3), and other non-invasive color quantification methods to study these poorly-understood ornaments in wild or naturally interacting birds.

Bare part colors can change in seconds. These quick-changing ornaments open up a world of new opportunities for students to quantify colors in real-time experiments using photographic and other non-invasive methods. For example, Crested Caracaras (*Caracara cheriway*) can rapidly change the color of the fleshy areas at the base of the beak, their ceres, from dark to light by reducing blood flow. Caracaras acting as aggressors have light-colored ceres, while recipients of aggression have dark-colored ceres (Dwyer 2014). Here, Dwyer visually documented color through a scope while watching >2,500 interactions between caracaras—a good reminder that excellent color science can be achieved without a spectrophotometer (which would not have been possible to use here without dramatically interfering with the natural social interactions of caracaras). See Bamford et al. (2010) for similar results in Lappet-faced Vultures *Aegypius tracheliotos*, who also flush their face rapidly in social situations. On the scale of days to week,

bills change color rapidly in response to different physiological conditions (e.g., in American Goldfinches *Spinus tristis* (Rosenthal et al. 2012) and King Penguin *Aptenodytes patagonicus* (Schull et al. 2016); see further work on penguin bill color in (Nolan et al. 2010; Schull et al. 2016).

Further, scientists have not considered all birds with equal fervor when quantifying coloration, and many species that need further study have bare part ornamentation. Consider raptors: they often have colored ornaments in their ceres and legs. For example, the social Black Kite *Milvus migrans* may use carotenoids in their legs and ceres to signal social status; here, the authors showed that visually assessing color with a color chart was repeatable and correlated with spectrophotometric measurements (Blas 2013). Seabirds, penguins, ratites, and more all have interesting bare part coloration that deserves further study—perhaps with non-invasive methods at a distance.

Bird eyes cover the whole rainbow of colors, but they have been poorly studied (Corbett et al. 2024). Corbett and colleagues (2024) systematically reviewed bird eye research to show that eyes that look similar to us—white eyes, yellow eyes, or red eyes—gain their color from a huge variety of pigments (Figure 1.5). Spectrophotometric methods typically use light that is too bright to safely probe eyes, so as above, photography is a good quantification option. Together, we suggest that students consider non-invasive, time-dependent research projects on bare parts coloration, including bills, feet, eyes, bill gapes, and other fleshy ornaments—particularly in understudied, non-passerine birds.

<< Figure 1.5 about here >>

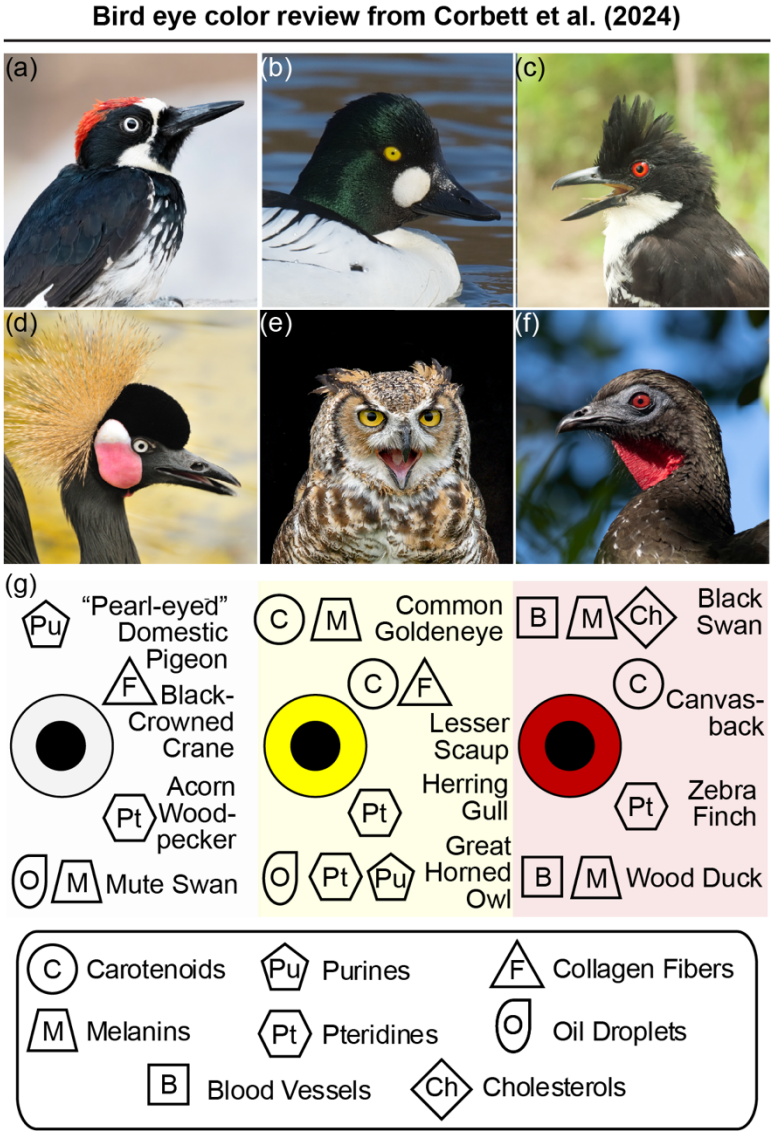

Figure 1.5. Bird eyes range widely in color, and many mechanisms cause these colors (Corbett et al. 2024). **(a-f)** Representative photographs illustrate three common iris colors in birds: (a,d) white, (b,e) yellow, and (c,f) red. **(a)** Acorn Woodpecker *Melanerpes formicivorus*. **(b)** Common Goldeneye *Bucephala clangula*. **(c)** Great Antshrike *Taraba major*. **(d)** Black Crowned Crane *Balearica pavonine*. **(e)** Great Horned Owl *Bubo virginianus*. **(f)** Crested Guan *Penelope purpurascens*. **(g)** Many different physical mechanisms produce the observed iris colors in white, yellow, and red-eyed birds (figure adapted from (Corbett et al. 2024)). Photo credits: (a, f) Marquette "Marky" Mutchler. (b) https://www.flickr.com/photos/sbern/, (CC BY 2.0 https://creativecommons.org/licenses/by/2.0/). (c) Diego Cueva. (d) Gregory Moine (CC BY 2.0, https://creativecommons.org/licenses/by/2.0/). (e) Peter K Burian (CC BY 4.0, https://creativecommons.org/licenses/by/4.0/deed.en). Data on mechanisms of bird eye color from Corbett et al. (2024).

1.4 How to measure reflectance: spectrophotometry, photography, and multi-/hyper-spectral imaging

1.4.1 Spectrophotometry

Today and for the past quarter century, the most common technique used to quantify bird color is **spectrophotometry**. Also called spectrometry or spectroradiometry, this describes a simple setup with a few components (Figure 1.6):

1) A **light source** illuminates the plumage surface
2) A **detector** collects the photons that reflect from a bird's integument
3) A **spectrometer** separates the reflected light into its constituent wavelengths to determine how much light is reflected at each wavelength
4) **Computer software** converts the spectrometer's output into a spreadsheet with one column for wavelength and one column for percent reflectance, which can then be plotted and analyzed.

You have many make and model options for each of the above components, and a few key considerations determine exactly what you are measuring. Most importantly, the geometry of the light source and the detector will determine *what* you are measuring (glossy or diffuse reflectance?). The light source and computer software are comparatively less impactful and typically come in a package deal with the detector and spectrometer that you select.

<<Figure 1.6 about here>>

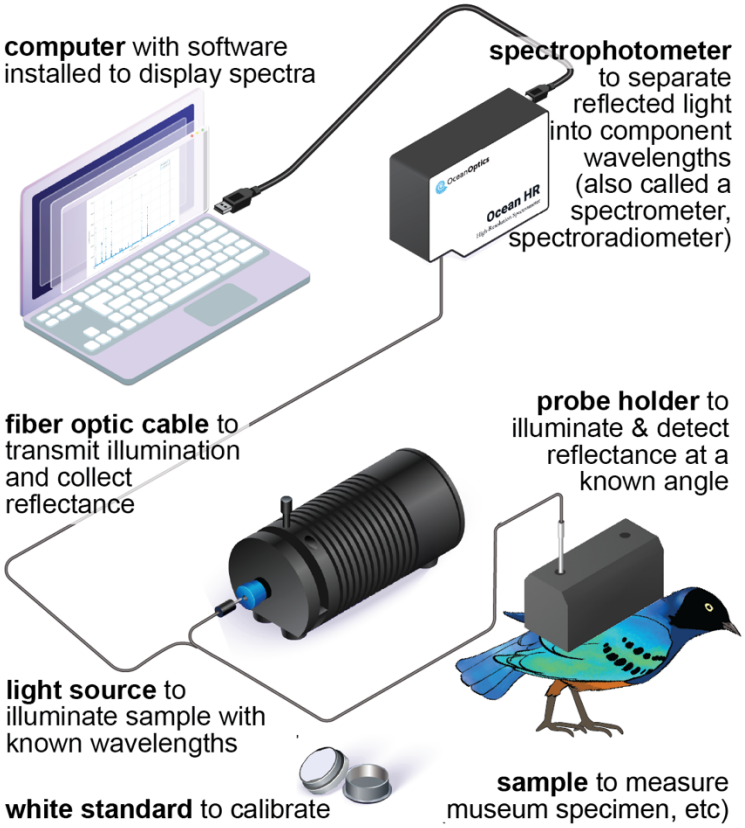

Figure 1.6: A typical spectrophotometry setup. Spectrophotometry artwork credit Ocean Optics; bird artwork credit Kay T. Xia.

1.4.1.1 Orientations of light source and detector

Scientists typically use one of four common geometries to orient the light source and the detector (Johnsen 2016). First, you can illuminate the sample at the same angle at which you collect reflected light (for example, normally incident at 90° or angled at 45°) using, usually, a reflectance probe. This probe is a bifurcated bundle of fiber-optic cables, some of which deliver light to the surface and the rest of which collect reflected light and direct it into the spectrometer. This can be very confusing at first, and typically one of the forks is labeled "to light source" and the other is labeled "to spectrometer". Make sure you connect each fork to its correct destination. Second, you can illuminate the sample at one angle and collect reflected light at 45° away from that angle, which requires a separate light source and detection probe. Third, you can place the sample inside (or flush with the port of) an **integrating sphere**. Integrating spheres are hollow balls where the inside is coated with a highly reflective material, such as Spectralon®, which diffusely reflects all light at roughly equal proportions. Then, as you illuminate your sample, the sphere collects all reflected light, and you can sample a small portion of that homogenous light

field at a certain angle. Often integrating spheres have a "gloss trap" you can activate if you wish to exclude all light that reflects in a mirror-like manner (specularly reflected light). Fourth, similar to #3, you can illuminate your sample with diffuse light that emerges from an integrating sphere and collect a small sample of the diffusely reflected light. Some companies like Ocean Optics make an illuminated integrating sphere for this purpose.

In all cases, you can, and perhaps should (depending on the ecology of your study species and the nature of your color(s) of interest), use more than one of the above geometries to assess how the colorful trait changes its reflectance depending on the angle of incident light and the viewing angle. You can purchase probe holders that hold your fibers at a predetermined angle, or you can 3D print your own (Figure 1.6). Generally, you can use whatever geometry you would like as long as you report it clearly. Good practice is to use the same geometry as other key papers in your subfield, if you would like to compare your results directly with those of other scholars. However, keep in mind that types of light sources and reflectance standards can impact your results, so the best way to compare colors across multiple species or individuals is to measure them with the same setup. Also, keep consistent the distance between your sample and your light source, the distance between your sample and the reflectance detector, and the integration time.

1.4.1.2 How to choose and use a light source

What range of wavelengths interests you? For most studies of bird plumage, one would ideally measure wavelengths from 300nm to 700nm, give or take 20 nm at either end. Many light sources include no or only weak illumination in the UV (300-400nm), which is why many studies of bird coloration report only visible reflectance (400-700nm). Generally a lamp should be broadband and diffuse. Pulsed xenon light sources, such as the Ocean Optics PX2, report spectral ranges from ~220-750 nm, a good range for avian color studies. Deuterium-halogen light sources, such as the Ocean Optics DH-2000, use two different bulbs to cover a very broad spectrum of ~200-2500 nm, which is far beyond what is needed but certainly usable. Tungsten-halogen lamps have less range into the UV, like the Ocean Optics HL-2000, which covers ~360-2400nm. For some lamps that use two bulbs, you may notice a discontinuity in your reflectance spectra at the wavelength range where one bulb's output decreases in power and the other increases.

For all light sources, especially those that use two different bulbs, you should allow the lamp to warm up for as long as needed before starting measurements: turn it on at least 15 minutes before you are ready to begin measurements. Otherwise, you will find that your spectrum drifts over the course of your measurements as the bulb heats up and reaches equilibrium. If you are worried that your lamp is drifting, you can frequently recalibrate using a reflectance standard.

1.4.1.3 Does it matter if I do spectrophotometry in the dark, under overhead lights, etc?

Generally speaking, it does not matter much what the ambient light conditions are when you perform spectrophotometry, because the probes and detectors that you use often block all or nearly all of the surrounding light. However, there are a few exceptions. If you are working with a geometry where your equipment does not block overhead lights – for example, where the detector is an inch away from the bird and fixed in place with brackets – then you should work in the dark. Many labs and departments have dark microscopy rooms for this purpose; alternatively, some ingenuity with opaque trash bags or boxes can achieve the same effect.

1.4.1.4 Types of spectrophotometers

Ornithologists most commonly use modular spectrophotometry systems, with separate light source, probe, detector, and spectrometer. Ocean Optics (formerly called Ocean Insight) and Avantes sell such systems, among other companies. An informal survey of ornithological studies of color from the last ten years suggest that Ocean Optics devices are most common.

A microspectrophotometer does just what it sounds like: measures spectral characteristics in microscopic samples, including reflectance, transmission, and/or absorption depending on the configuration. This is useful if you wish to quantify color of small parts of the integument or other very small features, such as retinal oil droplets. In an informal survey of recent literature, it seems that ornithologists often use a CRAIC technologies microspectrophotometer (e.g.,. Price-Waldman et al. 2025; Dunning et al. 2023) and sometimes design and construct their own (Hart 2004).

In many chemistry labs, absorbance spectrophotometers are used to measure the concentration of a substance (e.g. pigment) dissolved or suspended in liquid by transmitting light through a cuvette. For this purpose, fancy benchtop spectrophotometers integrate all of the above components in a precise geometry, into which a scientist can simply insert a cuvette. It is possible to use such a device for certain biological applications, particularly for flat or thin surfaces, but usually this is more difficult and not recommended for bird plumage or bare parts.

1.4.1.5 Report the full measurement geometry and configuration

When you write the Methods section of your manuscripts, be sure to report the full specifications and geometry of your spectrophotometry. That includes the exact equipment you used, the exact orientations, (very often missed) the integration time used, and (most often missed) the distance at which the probe is held from the plumage sample.

1.4.2 Digital photography

Digital cameras can, with a little work, be used to rigorously gather data on color. In fact, for those who are interested in the evolution of larger-scale integumentary patterns and patches,

photography can be an essential complement to spectrophotometry, which typically only considers reflectance at a single point (Mason and Bowie 2020).

Stevens et al. (2007), McKay (2013), Johnsen (2016), and Burns et al. (2017) list somewhat-solvable issues with using digital photography to measure bird coloration: cameras usually only gather data in three wavelength ranges (red, green, blue); most lenses do not permit UV light capture; cameras respond non-linearly to changes in light intensity; cameras may bias the resulting images toward certain wavelength ranges; cameras have post-processing software that changes images in sometimes-unknown ways; images are difficult to calibrate; and lighting is extremely variable (even when you use a diffuse flash). In short, recall that most consumer-available cameras are designed to produce images that look pleasing to the human eye—not unbiased images! For a camera to be unbiased, it should have a known relationship to the radiance of a scene so that images can be quantitatively analyzed using a camera's RAW output images, not JPG or otherwise (Akkaynak et al. 2014)). Modern cell phones apply a large amount of image processing that users cannot detect or undo; for that reason, it may be worth purchasing a DSLR (Digital Single-Lens Reflex) camera or pocket camera for more manual control. Some off-the-shelf cameras that scientists have used successfully include models of Sony A700, Canon EOS, and Canon Rebel (Akkaynak et al. 2014).

Here is a checklist of things to do to make digital photography usable for color analysis:

- If possible, configure your camera to take photographs of UV light as well as visible, possibly by taking two photos of every scene, one with a vis-block filter to block visible wavelengths and pass UV (having already removed the UV-blocking filter that most cameras have and equipping your camera with a lens that can transmit ultraviolet wavelengths; (Burns et al. 2017).
- Select a camera with manual white balance control and the ability to save TIFF/RAW file formats; see explanation and other useful camera characteristics in(Stevens et al. 2007; McKay 2013)
- Purchase reference calibration standards that show various known brightnesses of gray and various known hues, and include them in every photograph (e.g., X-rite calibration standards; Burns et al. 2017).
- Turn off white-point balancing and any other nonlinear transformation in your camera (Johnsen 2016; Stevens et al. 2007).
- Plan to use image processing software (e.g. imageJ) to extract red, green, and blue channel values for pixels or pixel ranges of interest, and calibrate these against your reference standard.
- Control your lighting environment as much as possible (e.g. selecting a bulb carefully for diffuse flash photography, or taking photographs in a controlled studio environment).
- If you are working in the field and cannot control the light environment, you can (i) include a color reference card in the frame of the photo for later analysis and processing

(most common) and/or measure the color and amount of ambient illumination using, e.g., devices that measure irradiance at each wavelength (uncommon and costly).

Many recent studies use digital photography to analyze large color patches in living birds/eggs in wild environments—all tasks that cannot easily be replicated using traditional spectrophotometry. While photography is, often, not as rigorous from a color quantification perspective as more analytical methods, it enables valuable eco-evo studies of wild birds and whole-body coloration. Plus, it is cheaper and sometimes easier. For example, researchers used photography in the wild to show that Semipalmated Plover eggs (*Charadrius semipalmatus*) have cryptic features which protect against predation; less-cryptic quail eggs placed into plover nests faced significantly higher predation than natural, more-cryptic plover eggs (Nguyen et al. 2007). Ornithologists also used photography to quantify plumage differences within and among wild bird populations: Atlantic forest birds have darker, more achromatic and less variable dorsal plumages compared to their ventral plumages (De La Torre et al. 2025); different subspecies of Song Sparrow (*Melospiza melodia*) in the San Francisco Bay area have quantitatively different plumage (Luttrell et al. 2015); and Horned Lark (*Eremophila alpestris*) plumage correlates with environmental conditions, enhancing camouflage and thermoregulation (Mason et al. 2023). Finally, Stoddard and colleagues showed that artificially colored stimuli in lab experiments can have unintended, variable effects depending on the animals' sensory system (Stoddard et al. 2019)— demonstrating how UV-vis photography can be combined with visual modeling to verify that what you are showing to animals matches what you want them to see. In many of these cited papers, authors also used spectrophotometry where possible.

1.4.3 Multispectral or hyperspectral imaging: the future of quantifying color?

More sophisticated cameras can capture a series of images that show precise reflectance at different ranges of wavelengths. Multispectral cameras usually capture four or so color channels, typically UV, blue, green, and red, using a series of filters attached to a classical camera. The resulting image shows high spatial resolution but low spectral resolution, since it typically only considers four different colors rather than a more continuous range (Hogan and Stoddard 2024).

For a much higher price tag, hyperspectral cameras use more sophisticated engineering to capture a stack of images with information about hundreds of color channels. As a result, you obtain a photograph where every pixel encodes a reflectance spectrum, where the X axis is wavelength. This is powerful technology, and it produces a massive dataset that can be challenging to know what to do with! Hyperspectral imaging has high spatial and spectral resolution, combining the advantages of photography with those of spectrophotometry(Hogan and Stoddard 2024). Typical spectrophotometry has high spectral resolution but low spatial resolution, and you must select and measure each point manually—a cumbersome and potentially biased process. Photography has high spatial resolution, and automatically samples

many points, but it has low spectral resolution. Hyperspectral imaging automatically captures spectral information at a huge number of spatial points, counteracting some downsides of spectrophotometry and photography.

Hyperspectral imaging is in its infancy for bird color research (Figure 1.7, Figure 1.8). Three studies show its high potential (Hogan and Stoddard 2024; Kim et al. 2012; McCoy and Prum 2019). Most recently, ornithologists used hyperspectral imaging, and wrote an analysis pipeline, to identify plumage differences, and the visual significance thereof, in King Bird-of-Paradise (*Cicinnurus regius*), Magnificent Bird-of-Paradise (*C. magnificus*), and the hybrid King of Holland's Bird-of-Paradise (*C. magnificus x C. regius*)(Figure 1.7, (Hogan and Stoddard 2024)). Using a Resonon Pika NUV imager, they showed that the many different plumage patches varied in the degree to which they were intermediate in the hybrid form, and that only some differences would be perceptible to a bird. The structural colors in the breast and tail were an intermediate form in the hybrid, which suggests interesting future research into the nanostructures and genetics underpinning those shiny colors. Importantly, the Bird-of-Paradise patches were not homogenous: therefore, hyperspectral imaging (which captures the entire patch) was a more useful method than typical spectrophotometry where several points are sampled. Hogan and Stoddard (2024) wrote an analysis pipeline to make sense of the large amount of data generated by hyperspectral imaging, and incorporate it into bird visual models, which will be immensely useful to future researchers.

<<Figure 1.7 about here>>

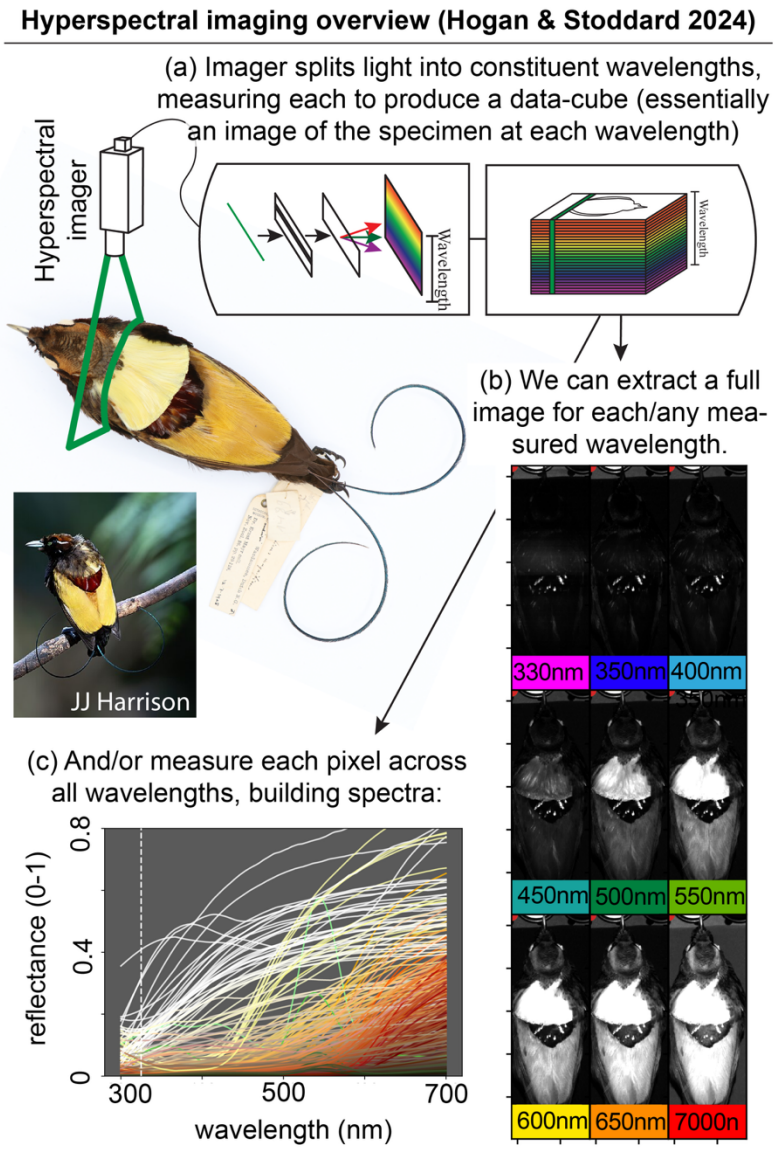

Figure 1.7. This figure is adapted from Hogan and Stoddard (2024), who wrote a hyperspectral analysis pipeline and applied it to King Bird-of-Paradise (*Cicinnurus regius*), Magnificent Bird-of-Paradise (*C. magnificus*), and the hybrid King of Holland's Bird-of-Paradise (*C. magnificus x C. regius*). Through hyperspectral imaging, **(a)** an imager produces a data cube where each slice is a high-resolution image of the specimen's reflectance at a given wavelength. Then the researcher can **(b)** extract an image for every wavelength measured and/or **(c)** extract a reflectance spectrum for any pixel in the image across all wavelengths, producing a rich dataset for further analysis. Inset image of Magnificent Bird-of-Paradise *Cicinnurus magnificus* n the wild credit JJ Harrison (CC BY-SA 4.0, https://creativecommons.org/licenses/by-sa/4.0/deed.en). Specimen images are courtesy David Ocampo. All data from Hogan and Stoddard (2024).

Kim et al. (2012) combined hyperspectral imaging with 3D scanning to produce a 3D spectral image of a specimen of the colorful Stella's lorikeet, *Charmosyna stellae*, using a rigorous custom-built setup (Figure 1.8). They also produced 3D maps of UV-fluorescence in the Northern Rosella *Platycercus venustus*, with yellow-green fluorescent back and belly contour feathers and blue-fluorescent crown, cheek, and wing (Figure 1.8). These high-resolution 3D spectral datasets are treasure troves of data on two parrots that are among the most colorful birds, demonstrating how whole-body analysis allows careful comparison of complete color patches. Further, their setup is a safe, repeatable, and comprehensive way to document UV-fluorescence in birds.

<<Figure 1.8 about here>>

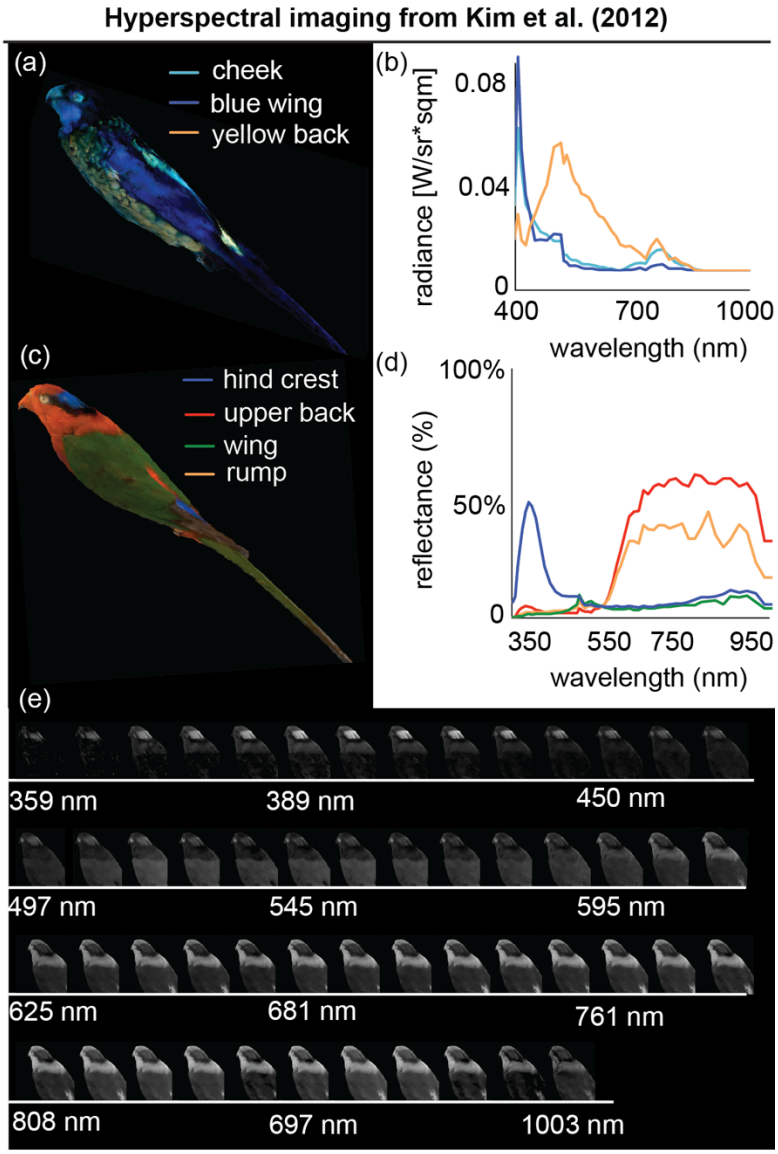

Figure 1.8. Kim and colleagues (2012) introduce a 3D scanning system paired with hyperspectral imager to comprehensively capture diffuse reflectance and fluorescence, demonstrated here in two parrots (data redrawn from (Kim et al. 2012)). **(a)** Northern Rosella *Platycercus venustus* UV-fluorescent plumage and its **(b)** fluorescent emission data (radiance). **(c)** Papuan Lorikeet *Charmosyna papou goliathina* 3D hyperspectral rendering, **(d)** diffuse reflectance readings (359nm–1μm) from the 3D hyperspectral patterns, and **(e)** full hyperspectral patterns where each image represents a single view of a comprehensive 3D hyperspectral image at a given wavelength. All data and images from Kim et al. (2012)

McCoy and Prum (2019) applied hyperspectral imaging to probe super black and bright color in individual, mounted feathers of the pale-billed Sicklebill *Drepanornis bruijnii* and the Splendid Starling *Lamprotornis splendidus* both before and after coating the feathers with gold for SEM. Here, hyperspectral imaging was useful because it could holistically capture within-feather variation from black to color (much like hyperspectral imaging is also useful to capture within-bird patch variation), as well as whole-feather color changes before and after a coating treatment. A Horiba and Cytoviva Model XploRA hyperspectral microscope was used.

Burns et al. (2017) predict that hyperspectral imaging may well become the new gold standard for ornithological research, particularly given its utility in field settings (e.g. in crabs; Russell and Dierssen 2015); I am inclined to agree. Hogan and Stoddard (2024) describe some future challenges and opportunities. Presently the largest barrier to using hyperspectral imaging is cost. A few challenges and opportunities include (i) deciding how to analyze the huge amount of data produced, (ii) accounting for the variable viewing geometries of scanning a 3D surface (Kim et al. 2012; Hogan and Stoddard 2024), (iii) algorithmically identifying patches from a hyperspectral dataset (rather than using human estimation of color patches); and—difficult to imagine now, but possible in theory—(iv) using hyperspectral imaging for moving, living animals in the wild (difficult because currently, it takes several minutes to scan an unmoving sample).

1.4.4 Processing photographs, whether digital or hyperspectral

Image processing tools have undergone an explosive radiation in the past 20 years. Surely between the time I write these words and the time this book goes to print, even more tools will be released. Once you have obtained a calibrated, linearized photograph, or a nice hyperspectral image stack, you can turn to any one of these open-source guides and processing packages (Hogan and Stoddard 2024; Troscianko and Stevens 2015; van den Berg et al. 2020; Mason and Bowie 2020; Chan et al. 2019).

Briefly, analyzing coloration from a classical or hyperspectral image involves two steps (Mason and Bowie 2020). First, you "segment" an image by selecting designated integumentary regions (groups of pixels) to analyze downstream. Second, you quantify color and other features of those selected regions, keeping in mind the visual system of your species of interest (see more in Chapter 2). Both steps can be achieved in many ways.

1.4.4.1 Segmenting an image: select interesting regions to analyze further

Regardless of the exact method used, by segmenting you aim to select regions of interest within which you would like to quantify color. Segmenting can be done by hand-drawing an outline over a region of interest on a bird, or by using automated tools ranging from simple pixel assignment to machine learning. For example, ornithologists can assign pixels based on simple "thresholding" (selecting all pixels based on a range of brightnesses) or more complicated k-means clustering, which minimizes the color distance between a pixel and the region's center (Mason and Bowie 2020). In a widely-cited and very useful publication, van den Berg et al. (2020) describe a full pipeline called Quantitative Colour Pattern Analysis (QCPA). When using QCPA, scientists can segment an image based on a sophisticated visual model of what a bird sees, incorporating both spatial acuity and color perception (van den Berg et al. 2020).

1.4.4.2 Quantifying color in the regions of interest

Generally, scientists wish to determine not only features of the color (hue, saturation, brightness) but also features of the spatial pattern (e.g., how sharp-edged it is; van den Berg et al. 2020). These methods are complicated and take into account research on avian visual systems (Chapter 2). For how-to guides and useful software, refer to van den Berg et al. (2020) as a starting point for digital photographs and Hogan and Stoddard (2024) for guidance and code to analyze hyperspectral images.

1.5 Key features of color and light

Now that you have measured reflectance using one or more of the methods above, I will discuss data analysis. Below I will describe commonly studied aspects of color (hue, saturation, and brightness) as well as less-studied features of reflected light (gloss, iridescence, and polarization).

<<Figure 1.9 about here>>

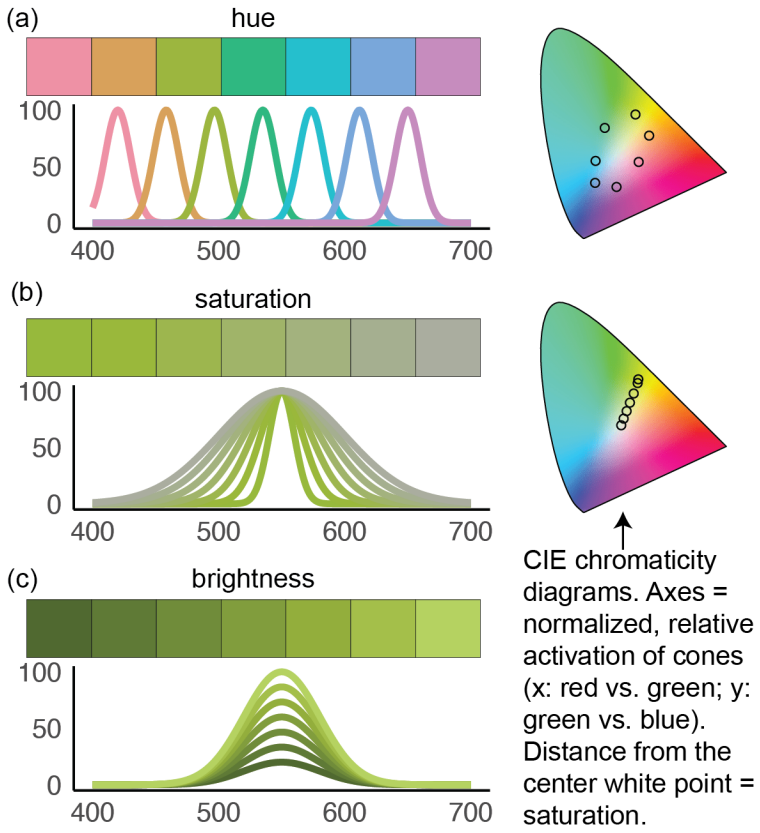

Figure 1.9. Variation in hue, saturation, and brightness illustrated using simulated reflectance spectra and (for hue and saturation) CIE chromaticity diagrams. Spectral reflectance curves (bottom panels of a-c) and corresponding color swatches (top panels in a-c) show how perceived color changes with **(a)** hue, **(b)** saturation (chroma), or **(c)** brightness. The CIE 1931 chromaticity diagrams (right panels of a-b) represent human-visible colors, where axes represent the relative activation of human cones, and the distance from the white center represents saturation. Note that hue, saturation, and brightness all depend on one another; for example, both saturation and brightness change in the spectra plotted in (b) and (c). Spectra were simulated as Gaussian reflectance functions, and colors were converted from CIE LCh (polarLUV) space to hex format to ensure perceptual uniformity. All analyses and visualizations were produced in R using ggplot2 for plotting (Wickham 2016), dplyr and tidyr for data wrangling (Wickham 2015; Wickham et al. 2020), colorspace (Zeileis et al. 2020) and pavo (Maia et al. 2019) for color format conversions and CIE plots, and gridExtra to assemble panels (Auguie et al. 2017).

1.5.1 Hue, saturation, and brightness: tristimulus color variables

Most commonly, researchers calculate a color's hue, saturation, and brightness based on the reflectance curve (Figure 1.9; (Montgomerie 2006)). **Hue** describes what we typically think of as color, or spectral location: is the plumage green, orange, or purple? You can calculate hue as a

simple physical property, without considering the sensory systems of a bird. In such cases, hue is the wavelength or wavelength range that contributes the most to the color, e.g., the wavelength at maximum reflectance for a roughly-Gaussian reflectance curve (Figure 1.9a). Hue can be represented in a 2D CIE xy chromaticity space, where the x- and y-axes indicate the relative contributions of the three human cone responses to a color (CIE stands for the French translation of International Commission on Illumination from 1931). Indeed, you can calculate hue while considering any receiver's sensory system, e.g., by determining the relative quantum catch of each category of color-sensitive cone cell in the eye. For certain plumages, the reflectance curve has two peaks and these classical calculations of hue do not make much sense (e.g., in UV+yellow colors or iridescent colors; (Montgomerie 2006)). In those two-peaked reflectances or other special cases, researchers should calculate hue based on the relative quantum catches of a receiver's cones (see details of this type of calculation in (Maia et al. 2019) and Chapter 2 herein).

Saturation refers to how pure a color is; to what extent it is composed of a single wavelength of light (Montgomerie 2006)? Saturation depends on how steep and narrow a color peak is (Endler 1990). A highly saturated green bird reflects a lot of green light in a very narrow wavelength range, and very little light of other colors. Less saturated colors look muddier, whiter, or greyer (Figure 1.9b). In a CIE colorspace, saturation is the distance along a line from the white point to the spectral locus (Figure 1.9b). People often use the term chroma interchangeably with saturation. Generally, you can calculate saturation as the reflectance in one region of the spectrum divided by the reflectance across the whole spectrum of interest– or any other calculation that approximates the same.

Brightness describes the total amount of light coming from a surface (Montgomerie 2006). A brighter plumage looks paler; a less bright plumage looks darker (Figure 1.9c). Note, however, that the perceptual phenomenon of brightness depends on features of animal vision: for example, a certain number of blue photons looks darker to us humans than the same number of yellow photons. Any calculation of brightness should compare the light reflected from a surface to a white standard. For example, one can calculate mean relative reflectance or integrate under the reflectance curve. Hue, saturation, and brightness all refer to radiance, not reflectance *per se*; bird integuments do not produce color independently of an illuminant. In practice, these values are extracted from color measurements with a known illuminant like a scientific light source.

Researchers use many related equations to calculate subtly different versions of hue, saturation, and brightness (see comprehensive table in (Montgomerie 2006)). The R package *pavo* provides useful guidance to extract metrics of color from reflectance curves (Maia et al. 2019); recall that "color" includes sensory experience, so it is important to include visual modeling in your work where relevant (see Chapter 2 herein). Consider a few examples of hue, brightness, and saturation calculations: to analyze how color changes with age in Tree Swallows *Tachycineta*

bicolor, researchers calculated hue as wavelength of maximum reflectance; blue chroma as reflectance from 400-512nm (the blue range) divided by total reflectance (from 300 to 700nm); and brightness as the average reflectance from 300 to 700nm (Bitton and Dawson 2008). Similar metrics were used in a study of white plumage in Collared Flycatcher (*Ficedula albicollis*)(Laczi et al. 2022); see subtly different calculations in an investigation of Rufous-capped Babbler (*Cyanoderma ruficeps*) (Fang et al. 2022). In a large study of *Tangara* tanagers, scientists calculated brightness as the mean relative reflectance; saturation as the wavelength of maximum reflectance minus the wavelength of minimum reflectance, divided by mean relative reflectance; and hue as the wavelength of maximum reflectance (Price-Waldman et al. 2025).

For carotenoid-colored feathers, reflectance does not follow a bell-curve shape but rather smoothly increases in a sigmoidal pattern. This complicates typical approximations of hue and saturation. For carotenoid-colored plumages, scientists can calculate a metric of hue as the wavelength at half-maximum reflectance and saturation as the left-width at half-maximum, or the wavelength range between the maximum and half-maximum reflectance values (see (McCoy et al. 2023; McCoy, Shultz, et al. 2021; Prum et al. 2014)).

Hue, saturation, and brightness depend on one another (Johnsen 2012). If you increase the concentration of carotenoid pigment in a feather, all three features change together: the hue gets redder, the color more saturated, and the brightness lower (McCoy et al. 2023). As a consequence, you should not treat these variables as independent in a statistical analysis of how color evolves.

In summary, any extracted feature of color, no matter how you calculate it, is simply a calculation. If you are interested in signal evolution, it is important to incorporate an animal's visual perception into your analysis. Some calculations accurately approximate a receiver's sensory experience; some calculations accurately approximate the quantity of pigment in an ornament; and some calculations do neither. Even if you try to optimize your metric for a relatively simple "true" quality of color, such as carotenoid pigment content, the best metric varies dramatically; Butler and colleagues showed that different metrics best predicted carotenoid content in red-colored ornaments of the House Finch *Carpodacus mexicanus*, Mallard Duck *Anas platyrhynchos*, and Zebra Finch *Taeniopygia guttata* (Butler et al. 2011). Butler and colleagues do reassure us that classic tristimulus measurements of hue and saturation are repeatable and trustworthy. For a complete treatment of how to interpret a bird's sensory experience of color, refer to Chapter 2.

1.5.2 Gloss and iridescence: appearance depends on angles

Iridescent birds are nature's gems. The hue of iridescent birds changes with angle, because these colors are produced by structural features that interfere with light in a wavelength-dependent

manner (see Chapter 7; (Prum 2006)). Male *Coeligena* hummingbirds flash brilliant purple, blue, and green patches that disappear into a matte black at sidelong angles (Giraldo et al. 2021). The breast-plate of Lawes' Parotia (*Parotia lawesii*) changes dramatically from yellow-orange or blue-green depending on the angle, thanks to stacked melanin rods inside the feather and a boomerang-shaped barbule cross section (Stavenga et al. 2011). Hummingbirds and birds-of-paradise perfected the art of iridescence, but many birds have angle-dependent hue thanks to internal and external feather morphology. Iridescence causes problems for spectrophotometry, because the geometry of your measurement really matters. Meadows and colleagues designed a repeatable setup for measuring iridescent colors in animals, mainly by rigidly controlling your geometry, rotating your sample, and quantifying how color changes with angle (Meadows et al. 2011). A goniometer can be used to carefully rotate your sample or your detection geometry.

The exact details of iridescence measurements vary, but the principles are the same: rotate your sample and look for angular changes in hue, including the angle at which the color is brightest or most saturated. For example, scholars measured iridescence across 80 bird species by detecting color over a range of angles (15°–135°) while the light source stayed fixed at 75° (Nordén et al. 2021). These authors showed that birds evolved nanostructures to unlock iridescence, roughly doubling the range of possible feather colors (Nordén et al. 2021). In another study, scientists tackled a new methodological challenge: measuring iridescence in the bill of the (aptly-named) Western Bluebill (*Spermophaga haematina*; (Nicolaï et al. 2024)). To avoid shadows created by the curved bill, they modified a goniometer to rotate both the sample and the probe, varying the light sources over eight different angles (from 55° to 90°; (Nicolaï et al. 2024)). In two different studies, scholars measured iridescence using a spectrometer with rotating fibers to detect reflectance between 10° and 50° away from the perpendicular over 5° increments: using this setup, scientists found that eggshells in Great Tinamous (*Tinamus major*) and feathers in manakins (*Lepidothrix isidorei, L. iris, L. nattereri and L. coeruleocapilla*) produce iridescent color (with air--keratin matrices in the manakin feathers and an unknown mechanism in the tinamou eggs). In principle, one can also measure the bi-directional reflectance function, in which reflectance is measured for all angles and azimuths of incident and reflected light.

Distinct from iridescence, **gloss** describes white specular highlights on certain birds. Glossiness is difficult to explain in words but easy to notice. Gloss is mirror-like reflectance from smooth surfaces; the opposite of matte; white highlights; and a sought-after component of human hair (Toomey et al. 2010; Maia et al. 2011). A House Sparrow’s matte black bib is not glossy, but a crow’s shiny plumage is (Toomey et al. 2010).

One way to quantify gloss is to calculate the ratio of specular to diffuse reflectance ((Hunter 1937), Figure 1.10). For example, in a study of many glossy black birds, scientists quantified specular reflectance with point-source spectrophotometry at 75° from the perpendicular and quantified diffuse reflectance with an integrating sphere that had a gloss trap to exclude mirror-

like, glossy reflectance (Maia et al. 2011). Maia and colleagues used those measurements plus feather anatomy analysis to show that gloss may be a less-ordered, weaker, less-chromatic form of iridescence– an evolutionary middle ground. In two further studies, scientists used roughly the same setup, except with probes oriented at 60°, to investigate gloss. First, in red-pigmented plumages, typically thought to be diffuse and directionless, larger, flatter barbs are glossier while curvier, smaller barbs are more diffusely-colored (Iskandar et al. 2016). Second, cassowary feathers demonstrated exceptional gloss thanks to a thick, smooth rachis (Eliason and Clarke 2020). In Eliason and Clarke's (2020) analysis of ratites, the glossiest plumage, on average, is Dwarf Cassowary (*Casuarius bennetti*) followed closely by Southern Cassowary (*Casuarius casuarius*); in comparison, Common Ostrich (*Struthio camelus)* is relatively matte (Figure 1.10).

Beyond canonically glossy birds like ravens, even apparently matte plumages can have substantial angle-dependent appearance. No visible highlights are obvious in super black feathers; however, super black Birds-of-Paradise are substantially darker from the viewing angle of a female (90°) and reflect far more light at low, sidelong angles due to their light-trapping geometry (McCoy et al. 2018).

<<Figure 1.10 about here>>

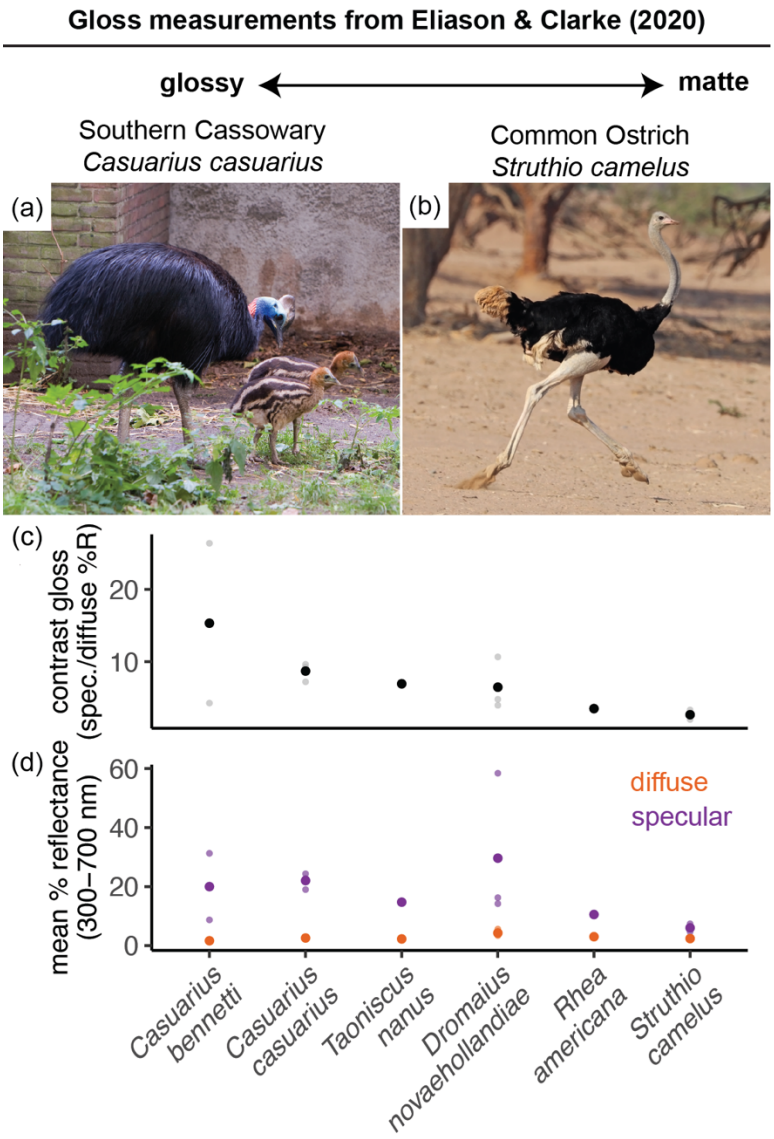

Figure 1.10. Ratites vary from exceptionally glossy to relatively matte (data from (Eliason and Clarke 2020)). **(a)** A Southern Cassowary *Casuarius casuarius* father with two chicks shows a glossy black plumage, with shiny highlights. **(b)** A Common Ostrich (*Struthio camelus*) looks comparatively dull. **(c)** Gloss is calculated as the percent specular reflectance divided by percent diffuse reflectance between 300 and 700 nm. Mean gloss is shown in black, while grey points represent the values for different plumage regions. **(d)** Reflectance was averaged between 300 and 700 nm; specular reflectance was measured at a 60° angle while diffuse reflectance was measured with an integrating sphere (Eliason and Clarke 2020). Smaller points represent the reflectance for different plumage regions, while the large points represent the mean value. Photo credits: (a) Charles J. Sharp (CC BY-SA 4.0, https://creativecommons.org/licenses/by-sa/4.0/) (b) Arjan Haverkamp (CC BY 2.0, https://creativecommons.org/licenses/by/2.0/). Data from Eliason and Clarke (2020)

1.5.3 Polarization

Before I define polarization, consider the familiar example of polarized sunglasses. Put on a pair of polarized sunglasses, and you'll see a crisper vista: maybe you will notice fish swimming beneath the surface of the ocean, which were previously hidden by the glare at the sea surface. Polarized sunglasses block most of the glare from smooth surfaces like water. Polarization refers to the plane in which an electric field wave (e-vector) oscillates (Warrant 2010; Johnsen 2012). Sunlight, and indeed most light sources of light, are collectively unpolarized, with many individual waves that area each polarized in random, different planes. But when sunlight reflects off of a smooth surface– like a highway, a smooth leaf, water, and perhaps a shiny bird–the reflected light often becomes linearly polarized in a direction parallel to the surface(Warrant 2010).

Light is often described in terms of linear or circular polarization, but all light can be described as elliptically polarized -- a combination of out-of-phase linearly and circularly polarized light waves (Brady and Cummings 2010). Linearly and circularly polarized light are two extremes. For linearly polarized light, the electric field vector oscillates in one plane perpendicular to the direction of propagation. For circularly polarized light, the electric field vector rotates as the wave propagates, either in a left-handed or right-handed direction (Brady and Cummings 2010; Warrant 2010). In nature, linearly polarized light is relatively common, while circularly polarized light is comparatively rare and may therefore produce a more discriminable signal (Marshall et al. 2019; Brady and Cummings 2010; Warrant 2010). Likewise, the degree to which light is polarized may itself be a useful component of colorful signals in animals (Marshall et al. 2019). You can divide any light into completely polarized and completely unpolarized and calculate the degree of polarization as the relative amount of each. Polarization is not a binary state (Johnsen 2012).

Do birds manipulate the polarization of light in their colorful signals? To my knowledge, nothing has been published on this topic. Many invertebrates produce colorful, polarized signals that they can detect and, in some cases, discriminate based on. For example, the Jewel Scarab Beetle *Chrysina gloriosa* reflects left-hand circularly polarized light (when illuminated with unpolarized light) thanks to helical nanostructures (Sharma et al. 2009). Further, this jeweled beetle also senses, and changes its behavior in response to, circularly polarized light (Brady and Cummings 2010; Warrant 2010). *Helioconus* butterflies recognize mates based on linearly polarized light (Sweeney et al. 2003). Stomatopod crustaceans produce, and sense, linearly polarized color signals and also circularly polarized color signals (Chiou et al. 2005; 2008). In these cases and likely many more, invertebrates use the polarization of light as a signal.

The question remains: do any birds use polarized light as a signal? First, can birds detect the polarization of light? It seems that they can; pigeons can be trained to behave differently depending on the polarization of light, and some birds seem to use polarization to navigate

(because linear polarization in the sky depends on the location of the sun; see (Åkesson 2024; Able 1989)). But whether birds can sense polarization with the acuity needed to respond to colorful signals (Johnsen 2012; Marshall et al. 2019) remains to be seen. Second, do birds have the right substrates to produce polarized signals? In principle, the keratin-air-pigmentary matrices that make up feathers can produce polarized light signals, just as beetles and stomatopods can. Further, any iridescent surface should reflect polarized light. Note: it is easy to detect whether a colorful signal preferentially reflects polarized light by using polarization filters and a light meter (Johnsen 2012). It is harder to show that animals can detect, and care about, the polarization of visual signals (Marshall et al. 2019)! To make a start, perhaps an enterprising PhD student should purchase a few polarization filters and visit the museum collection. The Birds-of-Paradise and hummingbirds might be a good first step!

Postscript: We humans are often described as incapable of sensing polarization. That is not strictly true. Some humans may be able to navigate based on patterns of polarization in the sky; Vikings used a birefringent calcite crystal– a sunstone-- to figure out the location of the sun when it had disappeared behind the horizon (Ropars et al. 2014). Many humans can detect Halidinger's brushes, a yellowish hourglass shape appearing in the center of our vision and indicating polarized light, with a little training. View a how-to guide here: (O'Shea et al. 2021).

1.6 Tetrahedral color space

The avian tetrahedral color space is a diagram that represents the hue and saturation of a color, plotted in a tetrahedral space where vertices mathematically represent four color-sensitive cone classes: red-, green-, blue-, and UV/violet-sensitive cones (Figure 1.4g-h, (Stoddard and Prum 2008; Endler and Mielke Jr 2005; Endler et al. 2005). A particular reflectance spectrum is plotted as a vector in the tetracolorspace, with spherical coordinates that define hue and saturation. The very center of the tetrahedron is the "achromatic point", and each plotted color is a vector emerging from that point. In tetrahedral colorspace, by convention, θ and φ define hue, and the distance r defines the saturation (see helpful explanatory figure in (Stoddard and Prum 2008)). The brightness of a color is not represented in a tetrahedral color space, which is instead best used to understand the hue and saturation of colors in birds. In a tetrahedral colorspace analysis, scientists can calculate many useful metrics (Burns et al. 2017): color span (patch-to-patch color distance); hue disparity (how different are the hue angles); color volume (the volume of the smallest polygon that encompasses all plumage measurements of interest); and more.

Indeed, a fantastic use of tetrahedral color spaces is to calculate the color gamut, or color space, occupied by a particular species or clade. For example, hummingbirds seem to occupy a larger color space than all other birds combined, based on a tetrahedral colorspace analysis ((Venable et al. 2022), Figure 1.4). Similarly, the plumage color diversity in a clade of neotropical tanagers seems to depend strongly on habitat: closed, versus open, habitats had more diverse colors (i.e., larger occupied colorspace volumes (Shultz and Burns 2013)).

Scientists also use the overlap between color polygons as a metric of color mimicry, showing, for example, that brood parasitic cuckoos successfully mimic their host's eggs (and hosts who more strongly reject eggs also must contend with more accurate mimics (Stoddard and Stevens 2011)).

1.7 Optical modeling

Ornithologists have increasingly turned to optical simulation tools to characterize and quantify color. After you measure the reflectance of a known ornament, optical models can help you understand what physical features produce that color under a range of illuminations, angles, and varying feature parameters. Further, optical models can help you test evolutionary hypotheses about how color evolves; e.g., you can simulate a wide variety of structural features and calculate the resulting reflectance for both observed and imagined scenarios (e.g., "in between" melanin granule shapes). Finally, optical models can calculate reflectance at specific angles that may be difficult to measure in the lab.

Scientists today can choose from many different optical simulation methods (for a decision flow-chart, see (McCoy, Shneidman, et al. 2021)). For example, for structures that are substantially larger than wavelengths of light, one can use ray-tracing—where lights are simulated as rays and wavelength-specific effects are usually ignored. McCoy, Feo, and colleagues (2018) used ray tracing to show that super black birds-of-paradise have vertically curved barbule arrays that cause a super black appearance (as low as 0.05% specular reflectance) by iteratively scattering and absorbing light. Further, these ray tracing models showed that super black birds look substantially brighter from sidelong angles; however, males habitually keep their best side oriented toward an observing female. For structures that approach the size of wavelengths of light, scientists typically use finite-element methods (FEM) or, more commonly in animal coloration research, finite-difference time-domain (FDTD) optical simulations. Note that sometimes you do not need FEM or FDTD simulation techniques at all; for example, multilayer modeling was sufficient to explain iridescence in male Peacocks (*Pavo cristatus*), which arises from two-dimensional photonic crystals of melanin, keratin, and air (Freyer et al. 2019).

A full treatment of optical simulation techniques is beyond the scope of this chapter. Here, we will provide a few examples of how researchers have used FDTD to understand bird coloration, by comparing simulation results to real reflectance curves. In short, FDTD simulations can help scientists test how certain structures or pigments contribute to the reflectance of a colorful ornament (among many other uses; (McCoy, Shneidman, et al. 2021), Figure 1.11). FDTD simulations solve for how the electromagnetic waves of light propagate through a known structure in a medium (such as a bird feather in air). A scientist may observe an unusually colorful bird ornament; measure its reflectance; and conduct electron microscopy to detect underpinning structures that may contribute to the color (Figure 1.11a). Then, they import that structure into simulation software, or draw an idealized version thereof, and define important

parameters: what illumination, background, refractive indices, etc. You can add monitors to calculate reflectance, transmission, and more (Figure 1.11b). Finally, you can analyze the output, in many cases reflectance curves, and compare them to reality, varying parameters such as feature size to better understand how the reflectance curves are produced, vary over different angles, and more (Figure 1.11c).

<<Figure 1.11 about here>>

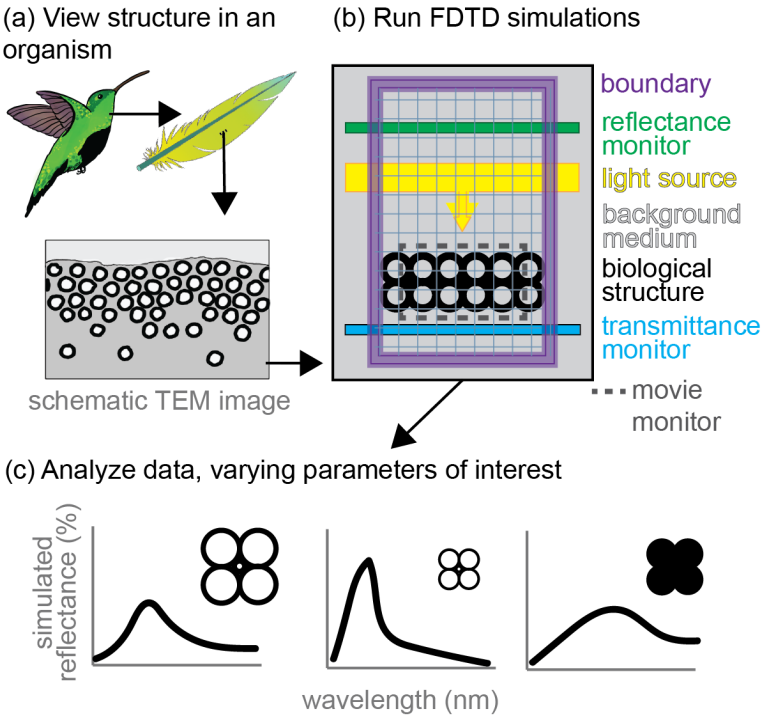

Figure 1.11. In a typical use of FDTD optical modeling (schematic adapted from McCoy, Shneidman, et al. (2021)), researchers **(a)** observe a colorful structure in an organism, measure the color using spectrophotometry, and identify possible structures and pigments that underpin the color (e.g., through transmission electron microscopy TEM). Next, **(b)** they can import the actual 2D or 3D structure into a simulation environment, and/or draw idealized versions of the structure, and then run the simulation. Finally, **(c)** they analyze the results of the simulation, including calculated reflectance curves; if desired, the researcher can compare outputs varying parameters of interest such as refractive index, size and shape of the elements, etc. Illustrations in (a) credit Kay T. Xia (hummingbird) and Ana Kimber (feather and TEM schematic).

FDTD has revealed much about bird coloration in the past 20 years. It allows scientist to link their experimental quantifications of feather color to structural causes and draw evolutionary conclusions. To give a remarkable example, consider the male Bird-of-Paradise Lawes's Parotia (*Parotia lawesii*), which has rainbow-iridescent feathers on its breast and nape (Figure 1.12a). Wilts and colleagues (2014) showed that this bird's breast feathers gain their omnidirectional, variable reflectance from layers of melanin rodlets in boomerang-cross-section-shaped feathers

(Figure 1.12). They found a strong match between simulated and measured reflectance (Figure 1.12f-g), strong evidence that the researchers successfully determined the physical basis of the feather's strange sparkle.

Similarly, researchers used FDTD to identify important structural drivers of color in a taxonomically wide range of birds with peculiar colors: dabbling ducks (tribe Anatini; two-part photonic heterostructures (Eliason and Shawkey 2012)); Wild Turkeys *Meleagris gallopavo* and Violet-backed Starlings *Cinnyricinclus leucogaster* (hollow melanosomes (Eliason et al. 2013)); pheasant *Polyplectron bicalcaratum* and pigeon *Patagioenas fasciata* (pigment granules that comprise a gradient of refractive indices(Eliason and Shawkey 2014); the blue wings and green heads of Mallard Ducks *Anas platyrhynchos* (multilayer periodicity and cortex thickness (Stavenga et al. 2017)); blue-green-purple iridescence in Common Magpie *Pica pica* (size and hollowness of melanosomes (Stavenga et al. 2018));red, orange, and yellow tanagers *Ramphocelus* spp. (microstructured barb and barbules; (McCoy, Shultz, et al. 2021); ultra white tail tips in Eurasian Woodcock *Scolopax rusticola* (disordered air-keratin nanostructures; (Dunning et al. 2023)); iridescent beak of Western Bluebill *Spermophaga haematina* (keratin-lipid multilayer "millefeuilles"; (Nicolaï et al. 2024)); and surely many more. FDTD may even allow us to quantify color in extinct avians, for whom any colors have long faded through fossilization; see, for example, evidence that a Cretaceous enantiornithine bird's head crest feathers may have been iridescent red to deep blue due to rod-shaped melanosomes (Li et al. 2025).

<<Figure 1.12 about here>>

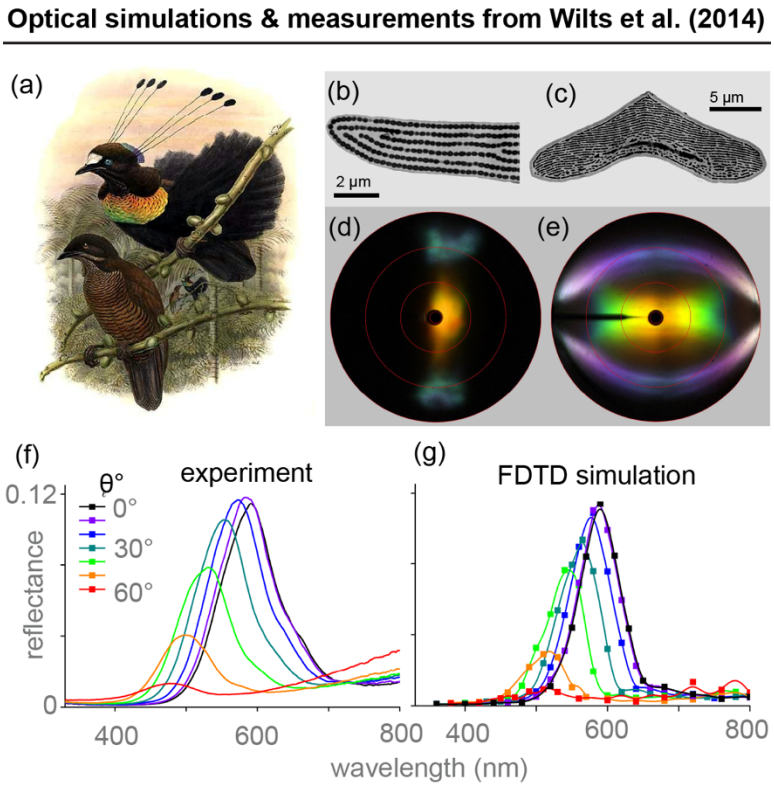

Figure 1.12. Wilts and colleagues (2014) used reflectance spectrophotometry and FDTD optical modeling to investigate sparkling feather reflections in a Bird-of-Paradise. **(a)** A male Lawes's Parotia *Parotia lawesii* displays dazzling multicolored breast feathers and nape feathers, framed by super black plumage, to attract a female. **(b)** TEM of an occipital feather barbule. **(c)** TEM of a breast feather barbule. **(d-g)** show the optics of breast feathers. **(d)** When a breast feather barbule is illuminated with a normal, ~5° aperture light beam, it scatters golden-yellow and cyan wavelengths of light over directions spanning roughly −60° to +60°. **(e)** When a breast feather is illuminated with a narrow-aperture, slit light source vertically and a 180° aperture horizontally, the barbule shows remarkable iridescence. **(f)** Measured reflectance spectra of breast feather barbules over angles θ = 0° - 60° (unpolarized light). **(g)** Simulated reflectance spectra calculated by FDTD modeling of breast feathers over angles θ = 0° - 60° (unpolarized light). Line colors in (f-g) are arbitrary and do not represent the colors perceived by a human or bird. Image credit in (a) Bowdler Sharpe, public domain; all other images and data from (Wilts et al. 2014).

1.8 Conclusion

Color seems like the simplest thing. Even a young child can name a chick yellow and a cardinal red. But color refers to the intersection of two of the most complex known phenomena: light and brains! Here, I have given an overview of the techniques used to quantify and analyze color. Over the last 20 years, ornithologists have perfected spectrophotometry to detect the reflectance of brilliant colors and open-source computer code to analyze it. Hyperspectral imaging and optical modeling, currently in their infancy for ornithological applications, show immense

promise for the future of quantifying color. Phenomena like gloss, bare part color, fluorescence, and more deserve further study, particularly in non-passerine birds. We humans have been noticing and studying bird color for centuries, and yet so much more discovery awaits.

1.9 Further reading:

Essential resources:

Johnsen, Sönke. The optics of life: a biologist's guide to light in nature. Princeton University Press, 2012.

Maia, Rafael, Hugo Gruson, John A. Endler, and Thomas E. White. "pavo 2: new tools for the spectral and spatial analysis of colour in R." Methods in Ecology and Evolution 10, no. 7 (2019): 1097-1107.

Quantifying color:

Johnsen, Sönke. "How to measure color using spectrometers and calibrated photographs." Journal of Experimental Biology 219, no. 6 (2016): 772-778.

Burns, Kevin J., Kevin J. McGraw, Allison J. Shultz, Mary C. Stoddard, and Daniel B. Thomas. "Advanced methods for studying pigments and coloration using avian specimens 1, 2." In The Extended Specimen, pp. 23-56. CRC Press, 2017.

Digital photography and hyperspectral imaging

Stevens, Martin, C. Alejandro Párraga, Innes C. Cuthill, Julian C. Partridge, and Tom S. Troscianko. "Using digital photography to study animal coloration." Biological Journal of the Linnean society 90, no. 2 (2007): 211-237.

van den Berg, Cedric P., Jolyon Troscianko, John A. Endler, N. Justin Marshall, and Karen L. Cheney. "Quantitative Colour Pattern Analysis (QCPA): A comprehensive framework for the analysis of colour patterns in nature." Methods in Ecology and Evolution 11, no. 2 (2020): 316-332.

Hogan, Benedict G., and Mary Caswell Stoddard. "Hyperspectral imaging in animal coloration research: A user-friendly pipeline for image generation, analysis, and integration with 3D modeling." PLoS biology 22, no. 12 (2024): e3002867.

Optical modeling

McCoy, Dakota E, Anna V Shneidman, Alexander L Davis, and Joanna Aizenberg. 2021. “Finite-Difference Time-Domain (FDTD) Optical Simulations: A Primer for the Life Sciences and Bio-Inspired Engineering.” Micron 151: 103160.

Acknowledgements

I thank Sönke Johnsen, Lorian Schweikert, and two reviewers for very useful feedback. I am very grateful to the authors of the studies who shared data and images that make up the figures herein, and to the photographers who allowed me to use their photographs.

Literature Cited

Able, Kenneth P. 1989. “Skylight Polarization Patterns and the Orientation of Migratory Birds.” *Journal of Experimental Biology* 141 (1): 241–56. https://doi.org/10.1242/jeb.141.1.241.

Åkesson, Susanne. 2024. “Polarization Vision in Birds.” In *Polarization Vision and Environmental Polarized Light*, edited by Gábor Horváth. Springer Nature Switzerland. https://doi.org/10.1007/978-3-031-62863-4_18.

Akkaynak, Derya, Tali Treibitz, Bei Xiao, et al. 2014. “Use of Commercial Off-the-Shelf Digital Cameras for Scientific Data Acquisition and Scene-Specific Color Calibration.” *JOSA A* 31 (2): 312–21. https://doi.org/10.1364/JOSAA.31.000312.

Andersson, Staffan, and Maria Prager. 2006. “Quantifying Colors.” *Bird Coloration, Volume 1*, 41–89.

Arnold, Kathryn E, Ian PF Owens, and N Justin Marshall. 2002. “Fluorescent Signaling in Parrots.” *Science* 295 (5552): 92–92.

Auguie, Baptiste, Anton Antonov, and Maintainer Baptiste Auguie. 2017. “Package ‘gridExtra.’” *Miscellaneous Functions for “Grid” Graphics* 9.

Bamford, Andrew J., Ara Monadjem, and Ian C. W. Hardy. 2010. “Associations of Avian Facial Flushing and Skin Colouration with Agonistic Interaction Outcomes.” *Ethology* 116 (12): 1163–70. https://doi.org/10.1111/j.1439-0310.2010.01834.x.

Berg, Cedric P van den, Jolyon Troscianko, John A Endler, N Justin Marshall, and Karen L Cheney. 2020. “Quantitative Colour Pattern Analysis (QCPA): A Comprehensive Framework for the Analysis of Colour Patterns in Nature.” *Methods in Ecology and Evolution* 11 (2): 316–32.

Bergeron, Zachary T., and Rebecca C. Fuller. 2018. “Using Human Vision to Detect Variation in Avian Coloration: How Bad Is It?” *The American Naturalist* 191 (2): 269–76. https://doi.org/10.1086/695282.

Bitton, Pierre-Paul, and Russell D Dawson. 2008. “Age-related Differences in Plumage Characteristics of Male Tree Swallows Tachycineta Bicolor: Hue and Brightness Signal Different Aspects of Individual Quality.” *Journal of Avian Biology* 39 (4): 446–52.

Blas, Julio. 2013. “Carotenoids and Skin Coloration in a Social Raptor.” *Journal of Raptor Research* 47 (2): 174–84. https://doi.org/10.3356/JRR-12-46.1.

Brady, Parrish, and Molly Cummings. 2010. “Differential Response to Circularly Polarized Light by the Jewel Scarab Beetle Chrysina Gloriosa.” *The American Naturalist* 175 (5): 614–20. https://doi.org/10.1086/651593.

Burns, Kevin J., Kevin J. McGraw, Allison J. Shultz, Mary C. Stoddard, and Daniel B. Thomas. 2017. “Advanced Methods for Studying Pigments and Coloration Using Avian Specimens.” In *The Extended Specimen: Emerging Frontiers in Collections-Based Ornithological Research*. CRC press. https://doi.org/10.1201/9781315120454.

Butler, Michael W., Matthew B. Toomey, and Kevin J. McGraw. 2011. “How Many Color Metrics Do We Need? Evaluating How Different Color-Scoring Procedures Explain Carotenoid Pigment Content in Avian Bare-Part and Plumage Ornaments.” *Behavioral Ecology and Sociobiology* 65 (2): 401–13. https://doi.org/10.1007/s00265-010-1074-1.

Casar, Jason R., Claire A. McLellan, Cindy Shi, et al. 2025. “Upconverting Microgauges Reveal Intraluminal Force Dynamics in Vivo.” *Nature* 637 (8044): 76–83. https://doi.org/10.1038/s41586-024-08331-x.

Chan, Ian ZW, Martin Stevens, and Peter A Todd. 2019. “PAT-GEOM: A Software Package for the Analysis of Animal Patterns.” *Methods in Ecology and Evolution* 10 (4): 591–600.

Chiou, Tsyr-Huei, Thomas W. Cronin, Roy L. Caldwell, and Justin Marshall. 2005. “Biological Polarized Light Reflectors in Stomatopod Crustaceans.” 5888 (August): 380–88. https://doi.org/10.1117/12.613117.

Chiou, Tsyr-Huei, Sonja Kleinlogel, Tom Cronin, et al. 2008. “Circular Polarization Vision in a Stomatopod Crustacean.” *Current Biology* 18 (6): 429–34. https://doi.org/10.1016/j.cub.2008.02.066.

Corbett, Eamon C., Robb T. Brumfield, and Brant C. Faircloth. 2024. “The Mechanistic, Genetic and Evolutionary Causes of Bird Eye Colour Variation.” *Ibis* 166 (2): 560–89. https://doi.org/10.1111/ibi.13276.

De La Torre, Gabriel Massaccesi, Victor Aguiar de Souza Penha, and Lilian Tonelli Manica. 2025. “Differences in Plumage Coloration between Ventral and Dorsal Regions on Atlantic Forest Birds.” *Ibis* 167 (3): 765–75. https://doi.org/10.1111/ibi.13383.

Dunning, Jamie, Antony W. Diamond, Stephen E. Christmas, et al. 2018. “Photoluminescence in the Bill of the Atlantic Puffin Fratercula Arctica.” *Bird Study* 65 (4): 570–73. https://doi.org/10.1080/00063657.2018.1563771.

Dunning, Jamie, Anvay Patil, Liliana D’Alba, et al. 2023. “How Woodcocks Produce the Most Brilliant White Plumage Patches among the Birds.” *Journal of the Royal Society Interface*, ahead of print, March 1. World. https://doi.org/10.1098/rsif.2022.0920.

Dwyer, James F. 2014. “Correlation of Cere Color with Intra-and Interspecific Agonistic Interactions of Crested Caracaras.” *Journal of Raptor Research* 48 (3): 240–47.

Eaton, Muir D. 2005. “Human Vision Fails to Distinguish Widespread Sexual Dichromatism among Sexually ‘Monochromatic’ Birds.” *Proceedings of the National Academy of Sciences* 102 (31): 10942–46. World. https://doi.org/10.1073/pnas.0501891102.

Eliason, Chad M., Pierre-Paul Bitton, and Matthew D. Shawkey. 2013. “How Hollow Melanosomes Affect Iridescent Colour Production in Birds.” *Proceedings. Biological Sciences* 280 (1767): 20131505. https://doi.org/10.1098/rspb.2013.1505.

Eliason, Chad M., and Julia A. Clarke. 2020. “Cassowary Gloss and a Novel Form of Structural Color in Birds.” *Science Advances*, ahead of print. https://doi.org/10.1126/sciadv.aba0187.

Eliason, Chad M., and Matthew D. Shawkey. 2012. “A Photonic Heterostructure Produces Diverse Iridescent Colours in Duck Wing Patches.” *Journal of the Royal Society, Interface* 9 (74): 2279–89. https://doi.org/10.1098/rsif.2012.0118.

Eliason, Chad M., and Matthew D. Shawkey. 2014. “Antireflection-Enhanced Color by a Natural Graded Refractive Index (GRIN) Structure.” *Optics Express* 22 Suppl 3 (May): A642-650. https://doi.org/10.1364/OE.22.00A642.

Endler, John A. 1990. “On the Measurement and Classification of Colour in Studies of Animal Colour Patterns.” *Biological Journal of the Linnean Society* 41 (4): 315–52.

Endler, John A, and Paul W. Mielke Jr. 2005. “Comparing Entire Colour Patterns as Birds See Them.” *Biological Journal of the Linnean Society* 86 (4): 405–31.

Endler, John A, David A Westcott, Joah R Madden, and Tim Robson. 2005. “Animal Visual Systems and the Evolution of Color Patterns: Sensory Processing Illuminates Signal Evolution.” *Evolution* 59 (8): 1795–818.

Fang, Yi-Ting, Cheng-Te Yao, Yu-Cheng Hsu, and Chih-Ming Hung. 2022. “Elevational Plumage Divergence in the Rufous-capped Babbler (Cyanoderma Ruficeps) on a Mountainous Island.” *Ibis* 164 (1): 151–67.

Freyer, Pascal, Bodo D. Wilts, and Doekele G. Stavenga. 2019. “Reflections on Iridescent Neck and Breast Feathers of the Peacock, Pavo Cristatus.” *Interface Focus* 9 (1): 20180043. https://doi.org/10.1098/rsfs.2018.0043.

Giraldo, Marco, Juliana Sosa, and Doekele Stavenga. 2021. “Feather Iridescence of Coeligena Hummingbird Species Varies Due to Differently Organized Barbs and Barbules.” *Biology Letters*, ahead of print, August 25. World. https://doi.org/10.1098/rsbl.2021.0190.

Hart, Nathan S. 2004. “Microspectrophotometry of Visual Pigments and Oil Droplets in a Marine Bird, the Wedge-Tailed Shearwater Puffinus Pacificus: Topographic Variations in

Photoreceptor Spectral Characteristics." *Journal of Experimental Biology* 207 (7): 1229–40.

Hogan, Benedict G., and Mary Caswell Stoddard. 2024. "Hyperspectral Imaging in Animal Coloration Research: A User-Friendly Pipeline for Image Generation, Analysis, and Integration with 3D Modeling." *PLOS Biology* 22 (12): e3002867. https://doi.org/10.1371/journal.pbio.3002867.

Hubbard, Joanna K, and Zachary WD Williard. 2023. "Spectra of Feather Samples Are Impacted by the Substrate Color against Which They Are Measured." *The Wilson Journal of Ornithology* 135 (1): 1–9.

Hunter, Richard S. 1937. "Methods of Determining Gloss." *NBS Research Paper RP* 958.

Iskandar, Jean-Pierre, Chad M Eliason, Tim Astrop, Branislav Igic, Rafael Maia, and Matthew D Shawkey. 2016. "Morphological Basis of Glossy Red Plumage Colours." *Biological Journal of the Linnean Society* 119 (2): 477–87.

Iverson, Erik N. K., and Jordan Karubian. 2017. "The Role of Bare Parts in Avian Signaling." *The Auk* 134 (3): 587–611. https://doi.org/10.1642/AUK-16-136.1.

Johnsen, Sönke. 2012. *The Optics of Life*. Princeton University Press.

Johnsen, Sönke. 2016. "How to Measure Color Using Spectrometers and Calibrated Photographs." *Journal of Experimental Biology* 219 (6): 772–78. https://doi.org/10.1242/jeb.124008.

Justyn, Nicholas M, Matthew J Powers, Geoffrey E Hill, Kayla Alexander, Adrián Naveda-Rodríguez, and Scott A Rush. 2023. "The Mechanisms of Color Production in Black Skin versus Red Skin on the Heads of New World Vultures." *Avian Research* 14: 100071.

Kim, Min H, Todd Alan Harvey, David S Kittle, et al. 2012. "3D Imaging Spectroscopy for Measuring Hyperspectral Patterns on Solid Objects." *ACM Transactions on Graphics (TOG)* 31 (4): 1–11.

Laczi, Miklós, Mónika Jablonszky, Gábor Markó, et al. 2022. "White Plumage Color as an Honest Indicator: Feather Macrostructure Links Reflectance with Reproductive Effort and Success." *Behavioral Ecology and Sociobiology* 76 (9): 125. https://doi.org/10.1007/s00265-022-03238-x.

Lay, Alice, Derek S. Wang, Michael D. Wisser, et al. 2017. "Upconverting Nanoparticles as Optical Sensors of Nano- to Micro-Newton Forces." *Nano Letters* 17 (7): 4172–77. https://doi.org/10.1021/acs.nanolett.7b00963.

Leertouwer, Hein L., Bodo D. Wilts, and Doekele G. Stavenga. 2011. "Refractive Index and Dispersion of Butterfly Chitin and Bird Keratin Measured by Polarizing Interference Microscopy." *Optics Express* 19 (24): 24061–66. https://doi.org/10.1364/OE.19.024061.

Li, Zhiheng, Jinsheng Hu, Thomas A Stidham, et al. 2025. “Iridescent Structural Coloration in a Crested Cretaceous Enantiornithine Bird from the Jehol Biota.” *eLife* 14 (August): RP103628. https://doi.org/10.7554/eLife.103628.

Luttrell, Sarah A. M., Sara T. Gonzalez, Bernard Lohr, and Russell Greenberg. 2015. “Digital Photography Quantifies Plumage Variation and Salt Marsh Melanism among Song Sparrow (Melospiza Melodia) Subspecies of the San Francisco Bay.” *The Auk* 132 (1): 277–87. https://doi.org/10.1642/AUK-14-107.1.

Maia, Rafael, Liliana D’Alba, and Matthew D Shawkey. 2011. “What Makes a Feather Shine? A Nanostructural Basis for Glossy Black Colours in Feathers.” *Proceedings of the Royal Society B: Biological Sciences* 278 (1714): 1973–80.

Maia, Rafael, Hugo Gruson, John A Endler, and Thomas E White. 2019. “Pavo 2: New Tools for the Spectral and Spatial Analysis of Colour in R.” *Methods in Ecology and Evolution* 10 (7): 1097–107.

Marshall, N. Justin, Samuel B. Powell, Thomas W. Cronin, et al. 2019. “Polarisation Signals: A New Currency for Communication.” *Journal of Experimental Biology* 222 (3): jeb134213. https://doi.org/10.1242/jeb.134213.

Martin, Rene P., Emily M. Carr, and John S. Sparks. 2025. “Does Biofluorescence Enhance Visual Signals in Birds-of-Paradise?” *Royal Society Open Science*, ahead of print, February. World. https://doi.org/10.1098/rsos.241905.

Mason, Nicholas A, and Rauri C K Bowie. 2020. “Plumage Patterns: Ecological Functions, Evolutionary Origins, and Advances in Quantification.” *The Auk* 137 (4): ukaa060. https://doi.org/10.1093/auk/ukaa060.

Mason, Nicholas A., Eric A. Riddell, Faye G. Romero, Carla Cicero, and Rauri C. K. Bowie. 2023. “Plumage Balances Camouflage and Thermoregulation in Horned Larks (Eremophila Alpestris).” *The American Naturalist*, ahead of print, February 1. Chicago, IL. https://doi.org/10.1086/722560.

McCoy, Dakota E, Teresa Feo, Todd Alan Harvey, and Richard O Prum. 2018. “Structural Absorption by Barbule Microstructures of Super Black Bird of Paradise Feathers.” *Nature Communications* 9 (1): 1–8.

McCoy, Dakota E, and Richard O Prum. 2019. “Convergent Evolution of Super Black Plumage near Bright Color in 15 Bird Families.” *Journal of Experimental Biology* 222 (18): jeb208140.

McCoy, Dakota E, Anna V Shneidman, Alexander L Davis, and Joanna Aizenberg. 2021. “Finite-Difference Time-Domain (FDTD) Optical Simulations: A Primer for the Life Sciences and Bio-Inspired Engineering.” *Micron* 151: 103160.

McCoy, Dakota E., Allison J. Shultz, Jacqueline E. Dall, Jennifer A. Dionne, and Sönke Johnsen. 2023. “The Carotenoid Redshift: Physical Basis and Implications for Visual Signaling.” *Ecology and Evolution* 13 (9): e10408. https://doi.org/10.1002/ece3.10408.

McCoy, Dakota E, Allison J Shultz, Charles Vidoudez, et al. 2021. “Microstructures Amplify Carotenoid Plumage Signals in Tanagers.” *Scientific Reports* 11 (1): 1–20.

McKay, Bailey D. 2013. “The Use of Digital Photography in Systematics.” *Biological Journal of the Linnean Society* 110 (1): 1–13. https://doi.org/10.1111/bij.12086.

Meadows, Melissa G, Nathan I Morehouse, Ronald L Rutowski, Jonathan M Douglas, and Kevin J McGraw. 2011. “Quantifying Iridescent Coloration in Animals: A Method for Improving Repeatability.” *Behavioral Ecology and Sociobiology* 65 (6): 1317–27.

Montgomerie, R. 2006. “Analyzing Colors.” In *Bird Coloration, Vol I, Mechanisms and Measurements*. Harvard University Press.

Nguyen, Linh P, Erica Nol, and Kenneth F Abraham. 2007. “Using Digital Photographs to Evaluate the Effectiveness of Plover Egg Crypsis.” *The Journal of Wildlife Management* 71 (6): 2084–89.

Nicolaï, Michaël P J, Gerben Debruyn, Mieke Soenens, Matthew D Shawkey, and Liliana D’Alba. 2024. “Nanoscale Millefeuilles Produce Iridescent Bill Ornaments in Birds.” *PNAS Nexus* 3 (4): pgae138. https://doi.org/10.1093/pnasnexus/pgae138.

Nicolaï, Michaël PJ, Matthew D Shawkey, Sara Porchetta, Ruben Claus, and Liliana D’Alba. 2020. “Exposure to UV Radiance Predicts Repeated Evolution of Concealed Black Skin in Birds.” *Nature Communications* 11 (1): 2414.

Nolan, Paul M., F. Stephen Dobson, Marion Nicolaus, Tim J. Karels, Kevin J. McGraw, and Pierre Jouventin. 2010. “Mutual Mate Choice for Colorful Traits in King Penguins.” *Ethology* 116 (7): 635–44. https://doi.org/10.1111/j.1439-0310.2010.01775.x.

Nordén, Klara Katarina, Chad M Eliason, and Mary Caswell Stoddard. 2021. “Evolution of Brilliant Iridescent Feather Nanostructures.” *eLife* 10 (December): e71179. https://doi.org/10.7554/eLife.71179.

O’Shea, Robert P., Gary P. Misson, and Shelby E. Temple. 2021. “Seeing Polarization of Light with the Naked Eye.” *Current Biology* 31 (4): R178–79. https://doi.org/10.1016/j.cub.2020.12.037.

Price-Waldman, Rosalyn M., Jarome R. Ali, Allison J. Shultz, Benedict G. Hogan, and Mary Caswell Stoddard. 2025. “Hidden White and Black Feather Layers Enhance Plumage Coloration in Tanagers and Other Songbirds.” *Science Advances*, ahead of print, July 25. World. https://doi.org/10.1126/sciadv.adw5857.

Price-Waldman, Rosalyn, and Mary Caswell Stoddard. 2021. “Avian Coloration Genetics: Recent Advances and Emerging Questions.” *Journal of Heredity* 112 (5): 395–416. https://doi.org/10.1093/jhered/esab015.

Prum, Richard O. 2006. “Anatomy, Physics, and Evolution of Structural Colors.” In *Bird Coloration: Mechanisms and Measurements*, vol. 1.

Prum, Richard O., Amy M. LaFountain, Christopher J. Berg, Michael J. Tauber, and Harry A. Frank. 2014. “Mechanism of Carotenoid Coloration in the Brightly Colored Plumages of Broadbills (Eurylaimidae).” *Journal of Comparative Physiology B* 184 (5): 651–72. https://doi.org/10.1007/s00360-014-0816-1.

Prum, Richard O., and Rodolfo Torres. 2003. “Structural Colouration of Avian Skin: Convergent Evolution of Coherently Scattering Dermal Collagen Arrays.” *Journal of Experimental Biology* 206 (14): 2409–29. https://doi.org/10.1242/jeb.00431.

Ropars, Guy, Vasudevan Lakshminarayanan, and Albert Le Floch. 2014. “The Sunstone and Polarised Skylight: Ancient Viking Navigational Tools?” *Contemporary Physics* 55 (4): 302–17. https://doi.org/10.1080/00107514.2014.929797.

Rosenthal, Malcolm F, Troy G Murphy, Nancy Darling, and Keith A Tarvin. 2012. “Ornamental Bill Color Rapidly Signals Changing Condition.” *Journal of Avian Biology* 43 (6): 553–64.

Russell, Brandon J, and Heidi M Dierssen. 2015. “Use of Hyperspectral Imagery to Assess Cryptic Color Matching in Sargassum Associated Crabs.” *PloS One* 10 (9): e0136260.

Schull, Quentin, F. Stephen Dobson, Antoine Stier, Jean-Patrice Robin, Pierre Bize, and Vincent A. Viblanc. 2016. “Beak Color Dynamically Signals Changes in Fasting Status and Parasite Loads in King Penguins.” *Behavioral Ecology* 27 (6): 1684–93. https://doi.org/10.1093/beheco/arw091.

Sharma, Vivek, Matija Crne, Jung Ok Park, and Mohan Srinivasarao. 2009. “Structural Origin of Circularly Polarized Iridescence in Jeweled Beetles.” *Science* 325 (5939): 449–51. https://doi.org/10.1126/science.1172051.

Shultz, Allison J., and Kevin J. Burns. 2013. “Plumage Evolution in Relation to Light Environment in a Novel Clade of Neotropical Tanagers.” *Molecular Phylogenetics and Evolution* 66 (1): 112–25. https://doi.org/10.1016/j.ympev.2012.09.011.

Stavenga, Doekele G., Casper J. van der Kooi, and Bodo D. Wilts. 2017. “Structural Coloured Feathers of Mallards Act by Simple Multilayer Photonics.” *Journal of the Royal Society, Interface* 14 (133): 20170407. https://doi.org/10.1098/rsif.2017.0407.

Stavenga, Doekele G, Hein L Leertouwer, N Justin Marshall, and Daniel Osorio. 2011. “Dramatic Colour Changes in a Bird of Paradise Caused by Uniquely Structured Breast Feather Barbules.” *Proceedings of the Royal Society B: Biological Sciences* 278 (1715): 2098–104.

Stavenga, Doekele G, Hein L Leertouwer, Daniel C Osorio, and Bodo D Wilts. 2015. “High Refractive Index of Melanin in Shiny Occipital Feathers of a Bird of Paradise.” *Light: Science & Applications* 4 (1): e243.

Stavenga, Doekele G., Hein L. Leertouwer, and Bodo D. Wilts. 2018. “Magnificent Magpie Colours by Feathers with Layers of Hollow Melanosomes.” *The Journal of Experimental Biology*, ahead of print. https://doi.org/10.1242/jeb.174656.

Stevens, Martin, C Alejandro Párraga, Innes C Cuthill, Julian C Partridge, and Tom S Troscianko. 2007. “Using Digital Photography to Study Animal Coloration.” *Biological Journal of the Linnean Society* 90 (2): 211–37.

Stoddard, Mary Caswell, Audrey E. Miller, Harold N. Eyster, and Derya Akkaynak. 2019. “I See Your False Colours: How Artificial Stimuli Appear to Different Animal Viewers.” *Journal of the Royal Society Interface Focus*, ahead of print, February 6. World. https://doi.org/10.1098/rsfs.2018.0053.

Stoddard, Mary Caswell, and Richard O Prum. 2008. “Evolution of Avian Plumage Color in a Tetrahedral Color Space: A Phylogenetic Analysis of New World Buntings.” *The American Naturalist* 171 (6): 755–76.

Stoddard, Mary Caswell, and Martin Stevens. 2011. “AVIAN VISION AND THE EVOLUTION OF EGG COLOR MIMICRY IN THE COMMON CUCKOO.” *Evolution* 65 (7): 2004–13. https://doi.org/10.1111/j.1558-5646.2011.01262.x.

Sweeney, Alison, Christopher Jiggins, and Sönke Johnsen. 2003. “Polarized Light as a Butterfly Mating Signal.” *Nature* 423 (6935): 31–32. https://doi.org/10.1038/423031a.

Thomas, Daniel B., Cushla M. McGoverin, Kevin J. McGraw, Helen F. James, and Odile Madden. 2013. “Vibrational Spectroscopic Analyses of Unique Yellow Feather Pigments (Spheniscins) in Penguins.” *Journal of The Royal Society Interface* 10 (83): 20121065. https://doi.org/10.1098/rsif.2012.1065.

Toomey, Matthew B., Michael W. Butler, Melissa G. Meadows, Lisa A. Taylor, H. Bobby Fokidis, and Kevin J. McGraw. 2010. “A Novel Method for Quantifying the Glossiness of Animals.” *Behavioral Ecology and Sociobiology* 64 (6): 1047–55. https://doi.org/10.1007/s00265-010-0926-z.

Troscianko, Jolyon, and Martin Stevens. 2015. “Image Calibration and Analysis Toolbox - a Free Software Suite for Objectively Measuring Reflectance, Colour and Pattern.” *Methods in Ecology and Evolution*, ahead of print. https://doi.org/10.1111/2041-210X.12439.

Venable, Gabriela X., Kaija Gahm, and Richard O. Prum. 2022. “Hummingbird Plumage Color Diversity Exceeds the Known Gamut of All Other Birds.” *Communications Biology* 5 (June): 576. https://doi.org/10.1038/s42003-022-03518-2.

Wails, Christy N., Eva D. Gruber, Ethan Slattery, Lucy Smith, and Heather L. Major. 2017. "Glowing in the Light: Fluorescence of Bill Plates in the Crested Auklet (Aethia Cristatella)." *The Wilson Journal of Ornithology* 129 (1): 155–58. https://doi.org/10.1676/1559-4491-129.1.155.

Warrant, Eric J. 2010. "Polarisation Vision: Beetles See Circularly Polarised Light." *Current Biology* 20 (14): R610–12. https://doi.org/10.1016/j.cub.2010.05.036.

Wickham, Hadley. 2015. "Dplyr: A Grammar of Data Manipulation." *R Package Version 04.* 3: p156.

Wickham, Hadley. 2016. *Ggplot2: Elegant Graphics for Data Analysis*. Springer-Verlag New York. https://ggplot2.tidyverse.org.

Wickham, Hadley. 2019. "Package 'Stringr.'" *Website: Http://Stringr. Tidyverse. Org, Https://Github. Com/Tidyverse/Stringr*.

Wickham, Hadley, Davis Vaughan, and Maximilian Girlich. 2020. "Tidyr: Tidy Messy Data. R Package Version 1.1. 3." *CRAN. R-Project. Org/Package= Tidyr*.

Wilkinson, Bradley P., Michael E. Johns, and Pete Warzybok. 2019. "Fluorescent Ornamentation in the Rhinoceros Auklet Cerorhinca Monocerata." *Ibis* 161 (3): 694–98. https://doi.org/10.1111/ibi.12715.

Williamson, Jessie L., Ethan F. Gyllenhaal, Selina M. Bauernfeind, et al. 2024. "Extreme Elevational Migration Spurred Cryptic Speciation in Giant Hummingbirds." *Proceedings of the National Academy of Sciences* 121 (21): e2313599121. https://doi.org/10.1073/pnas.2313599121.

Wilts, Bodo D, Kristel Michielsen, Hans De Raedt, and Doekele G Stavenga. 2014. "Sparkling Feather Reflections of a Bird-of-Paradise Explained by Finite-Difference Time-Domain Modeling." *Proceedings of the National Academy of Sciences USA* 111 (12): 4363–68.

Zeileis, Achim, Jason C Fisher, Kurt Hornik, et al. 2020. "Colorspace: A Toolbox for Manipulating and Assessing Colors and Palettes." *Journal of Statistical Software* 96: 1–49.