

# Shedding Light on Bird Egg Color: Pigment as Parasol and the Dark Car Effect

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**ABSTRACT:** The vibrant colors of many birds' eggs, particularly those that are blue to blue-green, are extraordinary in that they are striking traits present in hundreds of species that have nevertheless eluded evolutionary functional explanation. We propose that egg pigmentation mediates a trade-off between two routes by which solar radiation can harm bird embryos: transmittance through the eggshell and overheating through absorbance. We quantitatively test four components of this hypothesis on variably colored eggs of the village weaverbird (*Ploceus cucullatus*) in a controlled light environment: (1) damaging ultraviolet radiation can transmit through bird eggshells, (2) infrared radiation at natural intensities can heat the interior of eggs, (3) more intense egg coloration decreases light transmittance ("pigment as parasol"), and (4) more intense egg coloration increases absorbance of light by the eggshell and heats the egg interior ("dark car effect"). Results support all of these predictions. Thus, in sunlit nesting environments, less pigmentation will increase the detrimental effect of transmittance, but more pigmentation will increase the detrimental effect of absorbance. The optimal pigmentation level for a bird egg in a given light environment, all other things being equal, will depend on the balance between light transmittance and absorbance in relation to embryo fitness.

**Keywords:** bird eggs, solar radiation, thermoregulation, natural selection, biliverdin, ultraviolet.

Much of the success of evolutionary biology lies in its power to explain why organisms have the traits they do in functional terms (Williams 1966; Zimmer 2001; Losos 2011). Few traits are extravagant in appearance, well known even to those casually acquainted with nature, and present in hundreds of species but for which a general evolutionary explanation is lacking. The vibrant color of many birds' eggs is one of these curious traits. Hundreds of bird species lay eggs that are strikingly blue to green in color (Walters 2006; Hauber 2014), resulting from the predominant depo-

sition of the pigment biliverdin (Poole 1965; Baird et al. 1975; Burley and Vadehra 1989; Mikšík et al. 1996). Darwin invoked only the neutral explanations of ancestry and physiological by-product to explain blue-green egg coloration in domestic birds (Darwin 1868), but he knew that these explanations were insufficient: he asked August Weismann to take up the study, since he had achieved success with the bright colors of caterpillars (Darwin 1877). Subsequently, not Weismann but Alfred Russell Wallace and Alexander McAlldowie proposed the two main hypotheses—crypsis and photoprotection, respectively—that would be considered in the following century (McAlldowie 1886; Wallace 1889; Lack 1958; Fretwell 1973; Montevecchi 1976; Bakken et al. 1978; Oniki 1985). Both hypotheses suffered from a perceived lack of plausibility. Blue-green bird eggs are often laid in open nests and are thus exposed to predator view (Oniki 1985); however, they are generally highly perceptible, as they do not match nest backgrounds (Siefferman 2006; Stoddard et al. 2011). As for photoprotection, if its function were mainly to relieve heat stress (Romanoff and Romanoff 1949), blue-green pigment would seem unnecessary in the shaded environments in which such eggs are common (Lack 1958; Oniki 1979), especially in temperate areas where ambient temperatures are below optimal incubation temperatures; moreover, one might rather expect solar damage to favor white eggs since they are maximally reflective (Bertram and Burger 1981).

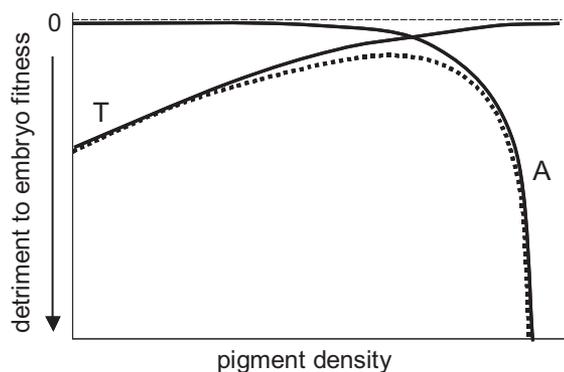
More recently, several other non-mutually-exclusive hypotheses have been proposed for blue-green egg coloration, but none has received consistent support or broad acceptance (Underwood and Sealy 2002; Kilner 2006; Maurer et al. 2011). A general feature of many recent explanations of blue-green egg color is their narrow range of applicability, relying on particular kinds of communication within or between species (e.g., Hanley et al. 2010; Soler et al. 2012; Duval et al. 2013). For example, a hypothesis to explain biliverdin-based coloration specifically based on sexual selection (Moreno and Osorno 2003) has been elaborated and tested with quantitative approaches in a number

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of species with mixed results (e.g., Hanley and Doucet 2009; Walters and Getty 2010; English and Montgomerie 2011); this hypothesis is relevant only for species where males provide parental care. Even if such signalling hypotheses do explain variation in blue-green color in certain species, we still require an explanation for why such coloration is widespread in birds more generally. In light of this situation, researchers have encouraged the elaboration and testing of more fundamental, nonsignalling hypotheses for bird egg coloration (Cassey et al. 2011; Maurer et al. 2011).

Here we develop and test an integrative hypothesis that revisits one of the oldest ideas for the function of eggshell coloration: photoprotection (McAldowie 1886). In particular, we propose that pigmentation—here predominantly from biliverdin—mediates a trade-off between two routes by which solar radiation can harm embryos (fig. 1): (1) transmittance through the eggshell and (2) absorbance leading to overheating (Lahti 2003, 2008).

The first route of potential damage to embryos is transmittance—the passage of light through the eggshell and into the egg. For instance, ultraviolet (UV) light, particularly in the UVB range (290–320 nm) and below, is known to have cytotoxic, mutagenic, and carcinogenic effects on vertebrate embryos (Setlow et al. 1993; Robert et al. 1996;



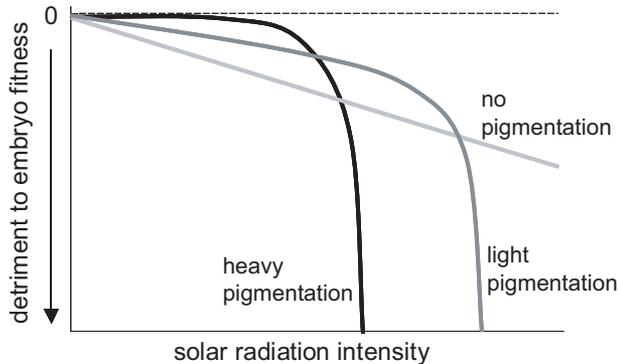
**Figure 1:** Hypothetical model illustrating a trade-off between two effects of pigmentation on the fitness of bird embryos subject to solar radiation. The solid lines represent the costs to embryonic fitness of a given amount of solar radiation at different eggshell pigmentation densities. Transmittance (T) generates the greatest cost at low pigmentation densities but generates no cost at high pigmentation densities because dense pigmentation makes eggshells opaque. Absorbance (A) generates negligible cost at very low pigmentation densities because little light is absorbed but generates greater costs at high pigmentation densities due to conduction of heat into the egg. Reflectance generates no cost. The heavy dotted line represents total fitness cost due to solar radiation. Thus, according to this hypothesis, as pigmentation density increases, two effects occur: pigmentation blocks light from transmitting (“pigment as parasol”), and absorbance creates heat buildup (“dark car effect”). In certain (moderate to high) light environments, the first effect will be beneficial, whereas the second effect will be detrimental.

Kouwenberg et al. 1999) and can reduce hatchability even after passing through the thick eggshells of chickens (*Gallus gallus*; Veterány et al. 2004). Even a highly reflective white egg does not reflect all light, and pigmentation might be adaptive if it blocks damaging light wavelengths from entering the egg. A poultry study showed that brown chicken eggshells are more efficient than white eggshells at blocking UV light from entering the egg (Shafey et al. 2002). Also notable is the fact that biliverdin has an absorbance peak in the UV, thus blocking light at those wavelengths particularly (Kennedy and Vevers 1973). Pigmentation as a protective barrier against light transmittance is also supported by a recent study of 74 bird species, where the average reflectance of eggshells (which varies inversely with pigmentation) correlated with the average amount of light transmitting into the eggs (Maurer et al. 2015). Damaging transmittance might not be restricted to the UV. The bird embryo, though translucent in early development (except for its pigmented eyes), might also absorb transmitted visible or infrared (IR) light and heat up or develop suboptimally as a consequence. Bakken et al. (1978) demonstrated that bird eggshells of various colors have particularly high IR reflectance compared to many other natural substances. Interestingly, the high IR reflectance of biliverdin in particular is not the case in the typical blues that vertebrates produce (Bakken et al. 1978), raising the possibility that the deposition of biliverdin on eggshells might originally have functioned in photoprotection.

The second route by which light might harm embryos is through absorbance. Light absorbed by an eggshell converts to heat. This heat can be lost to the environment by radiation and convection, but if heat production exceeds such loss, it will accumulate and conduct into the interior of the egg. Pigmentation decreases the reflectance of a surface and increases its absorbance—most noticeably in the visible range where this selectivity of reflectance creates color, but often also in the thermogenic IR wavelengths where nearly half of all solar radiation occurs (Gueymard 1995). Thus, pigmentation might contribute to egg heating in much the same way that the interior of a car heats in the sun to a greater extent if it is painted a dark color rather than a light color. Embryonic heating has two potential negative consequences. First, prior to incubation, temperatures above the threshold for embryonic development can reduce viability through incomplete development or microbial growth (Webb 1987; Cook et al. 2003, 2005). Second, heating of eggs after the onset of incubation can cause lethal and sublethal effects, especially since optimal development temperatures are generally within a few degrees of upper critical limits (Webb 1987; Reyna and Burggren 2012). The possibility for solar damage through overheating is heightened by the fact that a young embryo rests at the top of the egg, nearest the warmth of the parent and insulated from the cooler nest (McAldowie 1886; Turner 1997). Solar radi-

ation, therefore, need not heat the entire egg to damage an embryo. One prediction from the relationship between pigmentation and solar radiation is that darker eggs heat faster. This remains unclear: although manipulative studies have shown rapid increases in the temperature of darker eggs in sunlight, they used artificial and highly photoabsorptive paint (Montevecchi 1976; Bertram and Burger 1981; Magige et al. 2008). Such studies are therefore limited in their applicability (Ruxton 2012; Lahti 2015). The single study of unmodified eggs in nature found no difference among egg colors in heating rate (Westmoreland et al. 2007).

If both transmittance and absorbance of solar radiation can be damaging to embryos, this sets up the potential for a trade-off mediated by pigmentation: less pigmentation will increase any detrimental effect of transmittance, but more pigmentation will increase any detrimental effect of absorbance (fig. 1). In light environments where this trade-off operates, the optimal pigmentation level for an eggshell will depend on how transmittance and absorbance are best balanced in relation to embryo fitness (fig. 2). We test four key predictions from this hypothesis (table 1). The first two are underlying assumptions of the model: (1) UVB rays can transmit through bird eggshells, and (2) light at natural in-



**Figure 2:** Hypothetical model describing the relationship between variation in solar radiation and the fitness of bird embryos, assuming the relationship between pigmentation and embryonic fitness in figure 1. Each curve illustrates the detrimental fitness effect (Y-axis) when an egg initially at optimal fitness with respect to solar exposure (represented by the dotted line) is subjected to further, detrimental solar radiation for some constant amount of time at one of a range of intensities (X-axis). Absorbance of light by pigmentation shades the embryo from transmittance, resulting in higher fitness at moderate light intensities where the resulting heat can be lost to the environment by convection and radiation. However, there is a threshold light intensity for each degree of eggshell pigmentation, above which the rate of heat accumulation in the eggshell exceeds the rate of heat loss and embryo fitness decreases exponentially. Thus, the low absorbance of paler eggs results in their being optimal in the light environments of the highest intensity, whereas the high absorbance of darker eggs may render them optimal when light is at a moderate intensity.

intensities can heat the interior of an egg. The next two address whether pigmentation modulates these effects: (3) light transmittance is decreased by pigmentation (a phenomenon we call “pigment as parasol”), and (4) absorbance and heat accumulation are increased by pigmentation (a phenomenon we call the “dark car effect”). These predictions represent the mechanisms that would lead to a pigmentation-mediated trade-off. We also test whether it is predominantly absorbance or transmittance that leads to egg heating: in terms of the predictions in table 1, if P3a and P4a are both correct, then P3b and P4b conflict with each other. If the parasol effect includes not only the blocking of UVB transmittance but also the reduction of heating through the blocking of thermogenic IR transmittance, then pigmentation will be negatively correlated with temperature within the egg (P3b). On the other hand, if the dark car effect is relevant at the pigmentation levels of our study eggs at our experimental light intensities, such that eggs heat mainly through absorbance followed by conduction, then pigmentation will be positively correlated with temperature within the egg (P4b).

Our central question relates to egg color, but our hypothesis relates to pigmentation, and our methods are not biochemical but biophysical through tracking the fate of light in terms of spectral features and heating rates. We are therefore assuming, first, some relationship between pigment and color. This is uncontroversial, although the precise relationship between the two on an eggshell is not necessarily straightforward (Cassey et al. 2012; Hanley et al. 2015) and will eventually have to be determined empirically. Another assumption is that color—indicated by reflectance in the wavelength range of about 300–700 nm encompassing the vision of birds and their egg predators (Bennett and Cuthill 1994; Cuthill et al. 1999)—correlates with spectral features even more broadly. We test this assumption by measuring the reflectance, absorbance, and transmittance of eggshells between 250 and 800 nm, thus including some light in the damaging ultraviolet B and C (UVB-C, although UVC is not expected to penetrate the atmosphere) and the thermogenic near-infrared (IR) portions of the spectrum. We quantify the relation to color by assessing the correlation between reflectance in the visible wavelengths and the other spectral features we measure in different regions of the spectrum.

## Methods and Material

### Eggshells

Comparing eggs of various species would introduce unknown confounding factors, since eggs of different species vary not only in the intensity of pigmentation but also in the size, shape, eggshell thickness, and microstructure, and these can influence light dynamics (Maurer et al. 2015).

**Table 1:** Components of a model whereby bird egg color modulates the influence of solar radiation on embryonic fitness, including predictions to be tested in this study

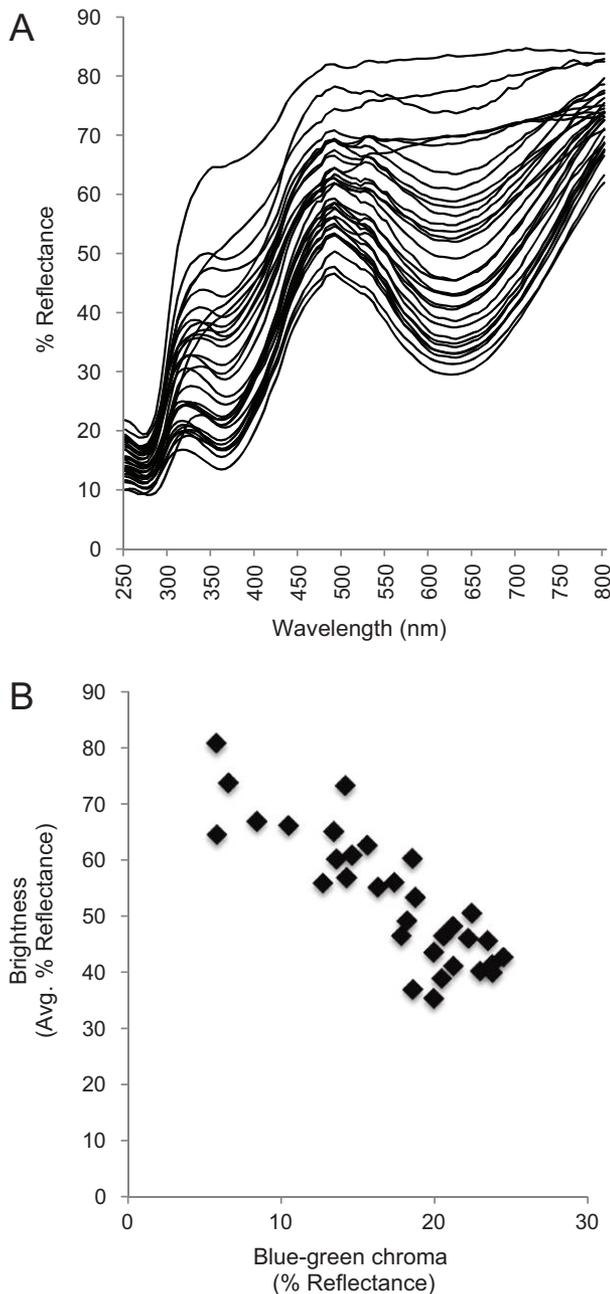
Hypothesis	Proposed mechanism	Prediction
H1: (Potential for) UV damage to embryos	UV radiation causes mutation or tissue damage	P1: UVB light at natural intensities transmits through eggshells
H2: (Potential for) solar heating of embryos	Heat accumulation causes developmental abnormality, bacterial growth, or hatching asynchrony	P2: IR light at natural intensities heats the egg interior
H3: Pigment as parasol	Pigmentation shields embryos from solar radiation	P3a: More intense egg coloration decreases light transmittance through eggshells, including at damaging UVB and thermogenic IR wavelengths P3b: (If heating by transmitted light is a factor) increased transmittance increases heating of the egg interior
H4: Dark car effect	Pigmentation absorbs heat that conducts into the egg	P4a: More intense egg coloration increases absorbance of light by the eggshell P4b: Increased absorbance increases heating of the egg interior

Therefore, our sample is comprised of eggs of varying color from a single species, the African village weaverbird *Ploceus cucullatus*. Village weaver females in a single locality lay eggs of varying color and maculation patterns between individuals, a diversity that has been found to relate to brood parasitism by the egg-mimicking diederik cuckoo *Chrysococcyx caprius* (Lahti and Lahti 2002; Lahti 2005). Thirty-two weaver eggs from the Gambia, South Africa, Mauritius, and the Dominican Republic were chosen specifically for their variation in color (from white to medium blue-green; see fig. 3A) and for the absence of spotting, so that spots would not introduce a confounding effect on spectral or thermal characteristics. Eggs were selected such that, to the human eye, white, light blue-green, and medium blue-green eggs were equally represented, and no locality was predominantly represented in any color class. White eggs were not found in the Dominican Republic, but otherwise, eggs of each color class were present in all locations. All eggs were collected by D. C. Lahti from the wild between 1999 and 2001 during the early incubation period (within 4 days of laying), blown empty, and stored in a lightless environment since that time in the bird collection at the University of Michigan Museum of Zoology. All 32 eggs were used in all analyses, except that IR irradiance measurements of three eggs were lost. Since egg albumen is nearly transparent, with a transmittance of 91.5% (Romanoff and Romanoff 1949), and bird embryos are positioned by chalazae close to the top of eggs such that relatively little albumen intervenes, eggshells form the most important barrier to light radiation at any wavelength (Romanoff and Romanoff 1949). For this reason, and because substitutive materials might have spectral and insulative properties not shared by albumen, we used empty eggshells

for all tests. Since bird egg albumen has a thermal conductivity roughly 10 times that of air (0.238 W/m<sup>2</sup>·°C for albumen [Kong et al. 2014] compared to 0.026 W/m<sup>2</sup>·°C for air at 25°C), any effects of light absorbance on internal egg temperature will be underestimated here, rendering our tests conservative.

#### *Lighting, Spectroscopy, Thermal Dynamics, and Data Analysis*

We irradiated eggshells with three different light sources, depending on whether we were testing hypotheses related to ultraviolet (UV), human visible (VIS), or infrared (IR) radiation. For UV light, we used a 12.7-cm-long, 20W 125V compact fluorescent UVB reptile bulb with advertised power 50  $\mu$ W UVB at 18-in distance (Zilla 11410 Desert 50). For VIS light, we used a 26.7-cm-long, 105W 125V compact fluorescent bulb (CFS32; US Way Lighting), which produces 6,720 lumens at a color temperature matching that of mean daylight at 6,500 K (Judd et al. 1964). For IR light, including all heating tests, we used a 16.7-cm-long, 250W 125V incandescent-reflector clear-bulb infrared heat lamp (R40 shape, C9 filament, 2,200 lumens; General Electric). Each bulb was placed within a metallic cone reflector and suspended over the sample eggs. The VIS and IR bulbs were positioned such that the base of the bulb was 125 cm above the eggs, producing irradiance at all wavelengths between 250 and 800 nm, well below typical direct solar levels (Navarro and Lahti 2014). The UV bulb, being much less intense than the other two bulbs over the target wavelengths (UVB: 290–320 nm), was placed at a height of 25 cm, which we determined by spectrophotometric measurements of absolute irradiance to be the lowest height at which all eggs



**Figure 3:** Reflectance spectra (A) and relationship between brightness and chroma (B) of the 32 eggs used in this study. Eggs ranged from a medium blue-green (lower curves in A, with a reflectance peak around 500 nm) to white (upper curves in A, with high overall reflectance). The higher the average reflectance of eggs (brightness), the less blue-green chroma they exhibited, as represented by the height of the blue-green reflectance peak (B).

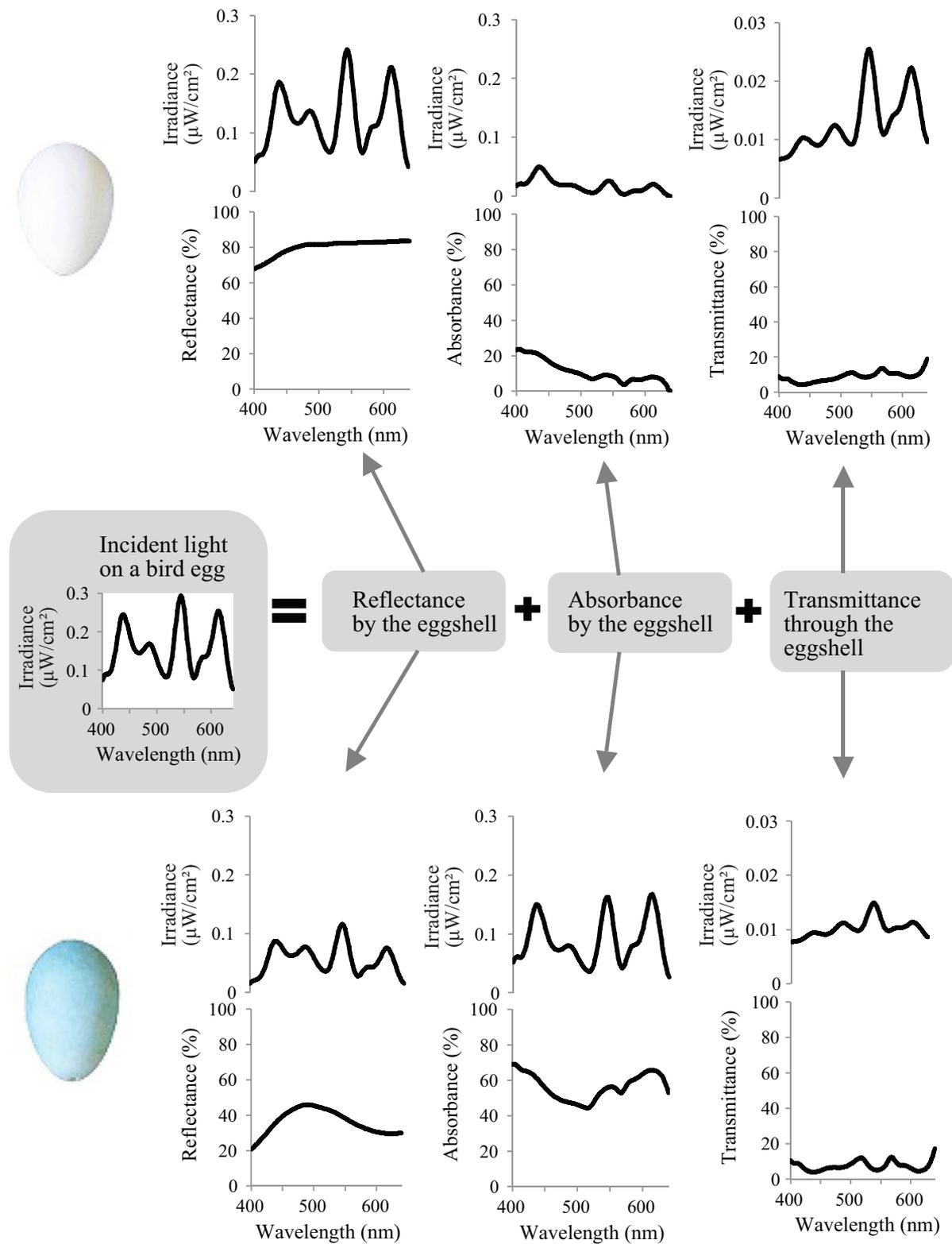
in the sample would be irradiated identically ( $\pm 0.001 \mu\text{W}/\text{cm}^2$ ). All eggs were also identically irradiated by the VIS and IR bulbs. Since all bulbs were much less intense than direct sunlight in all regions of the spectrum, and as we were

interested primarily in comparing egg colors in relation to the fates of light including heating, our results often focus on relative rather than absolute values.

Spectral data were gathered with an Ocean Optics USB2000 UV-VIS spectrophotometer and associated Spectrasuite software (Ocean Optics, Dunedin, FL). Reflectance was measured with a 200-Hz pulsed xenon light source (Ocean Optics PX-2) and a 400- $\mu\text{m}$  reflection probe (Ocean Optics R400-7) held perpendicular to the eggshell at 8 mm distance under an opaque cloth. Integration time was set at 250 ms. Reflectance measurements were standardized with a diffuse polytetrafluoroethylene tile that reflects  $>98\%$  of light over all sampled wavelengths (Ocean Optics WS-1). Reflectance measurements were taken immediately before irradiation with the light sources. Irradiance measurements were taken with a custom 1-mm bare fiber inserted 7 mm from beneath the stage supporting the sample into the side of each eggshell. Eggs ranged from 14.6 to 16.4 mm wide, so the fiber extended to roughly the center of each egg. Absolute irradiance measures were obtained by calibrating the power of the experimental light at each wavelength to that of a known power source (Ocean Optics DH2000-CAL deuterium tungsten-halogen). We derived percent transmittance by subtracting the irradiance detected by a bare fiber within an eggshell from the irradiance detected by that same bare fiber in the same location but with the eggshell removed. Given the reflectance ( $R$ ) and transmittance ( $T$ ) percentages, absorbance by the eggshell was calculated as  $A = 100 - (T + R)$ .

Within the overall spectral range of 250–800 nm, our predictions highlight three spectral regions of particular interest: (1) Harmful UV rays that are not entirely absorbed by the ozone layer and thus can enter and harm living cells spanning 290–320 nm. UVB radiation is represented by average irradiance in this wavelength range. (2) The intensity of coloration of each eggshell is represented in two ways: average reflectance (brightness or brilliance) in the human-visible spectrum (400–700 nm) and the height of the distinctive curve that represents blue-green chroma (Lahti 2008; Navarro and Lahti 2014), calculated as  $R_{500} - (R_{400} + R_{650})/2$ , where the  $R$  subscripts indicate wavelengths in nanometers. Nonwhite eggs varied negligibly in hue (i.e., the location of peak reflectance within this curve was 489–492 nm for any egg that peaked between 400–650 nm; fig. 3A). Finally, (3) thermogenic near-IR radiation was represented by average irradiance between 700 and 800 nm. We used general linear models to test for an effect of the intensity of eggshell coloration (blue-green chroma) on the fate of light incident on the eggshell in the UVB, VIS, and IR regions separately.

The effect of eggshell color on heating was tested by artificially exposing each egg to the IR light source at 125 cm height. We placed each egg on its side, with a bare wire thermocouple



**Figure 4:** Three fates of visible light incident on a bird eggshell, illustrating the influence of eggshell color. A broad-spectrum (400–650 nm) fluorescent light source with a near-solar color temperature (6,500 K) illuminated eggs from a height of 125 cm to yield an absolute irradiance over its effective spectrum, as graphed in the gray box. Graphs of reflectance (R; left column), absorbance (A; middle column), and trans-

(Omega engineering type T 36AWG  $\pm$  0.1°C) inserted into the eggshell from beneath through a drilled hole so that its tip extended to just below the exposed side of the eggshell. Data were recorded every 1 s into a Hobo U12-14 thermocouple datalogger. Each egg was exposed twice, except for two eggs where thermocouples shifted during trials; repeatability was high ( $F_{63,64} = 7.6, P < .01, r = 0.91$ ; Lessells and Boag 1987). We calculated the rate of heating of each egg ( $T_{eq}$ ; Voss and Hainsworth 2001):  $y = \ln[(T_{Et} - T_{E\infty}) / (T_{E0} - T_{E\infty})]$ , where  $T_{Et}$  = temperature at a given point in time,  $T_{E0}$  = initial temperature, and  $T_{E\infty}$  = asymptotic temperature. We averaged the values from the two trials. The second-order time constant, K2, represents the egg's heating rate once it begins gaining heat and is estimated as the derivative of the slope of the heating curve. Heating rate measurements of eggs were compared to those of bare thermocouples as a baseline. Data are available from the Dryad Digital Repository: <http://dx.doi.org/10.5061/dryad.21723> (Lahti and Ardia 2016).

## Results

### *Spectral Characteristics of Sample Eggs*

The 32 village weaver eggs used in this study ranged from medium blue-green to white. The more blue-green eggs had a lower average reflectance, with more variation in reflectance across wavelengths, including a small (~15%–40% reflectance) peak centering around 305 nm and a broader and higher (~40%–65% reflectance) peak centering around 500 nm. The whiter eggs had a higher overall reflectance, resulting in a general flattening of the peaks (fig. 3A). Brightness (average visible reflectance) was strongly negatively correlated with blue-green chroma in our sample of eggs (fig. 3B); accordingly, all results reported here for brightness are qualitatively similar but opposite in sign to those analyzed in terms of blue-green chroma. A graphical example of relevant spectral results is presented in figure 4, including relative (reflectance, transmittance, and absorbance) and absolute (irradiance) measurements for two sample eggs, one medium blue-green and one white. Correlations among reflectance, absorbance, and transmittance within and across spectral regions are presented in figure 5. Several generalities are evident from these correlations. Each of these three fates of light is strongly correlated across spectral regions; trans-

mittance is somewhat less strongly correlated across regions than are the other two, probably because the much lower transmittance values increase the effect of measurement error. Reflectance and absorbance are strongly negatively correlated with each other; this was not surprising, as values for transmittance are comparatively small, and all three proportions sum to 1. Transmittance is positively correlated with reflectance and negatively correlated with absorbance, although only weakly in the UV. Finally, correlations between light dynamics in the VIS and IR regions are stronger than those between UVB and VIS.

### *P1: UVB Light at Natural Intensities Transmits through Eggshells*

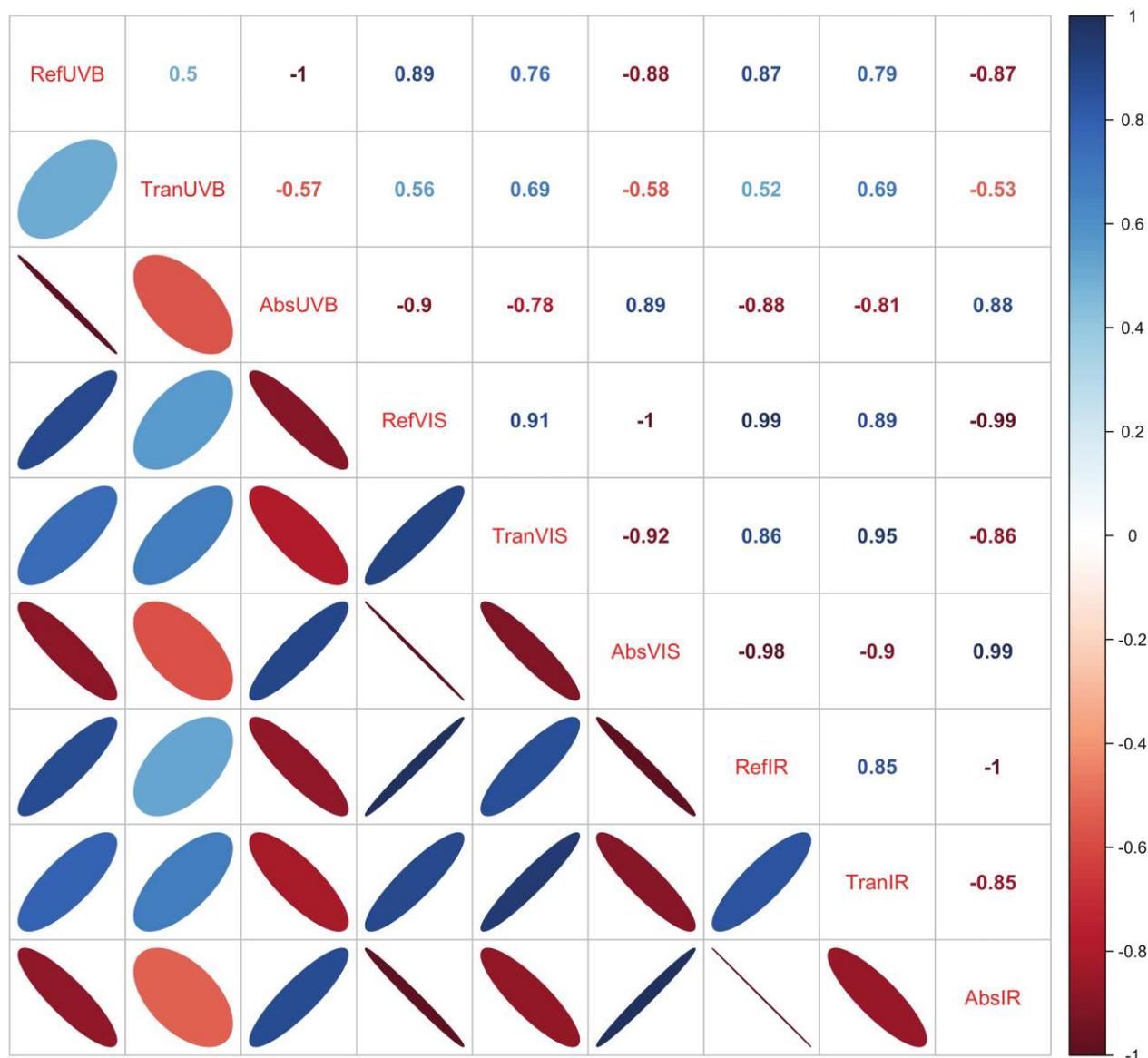
This prediction was supported: eggshells permitted transmittance of UV light, although much attenuated and varying by wavelength. The percentage of incident UV light that transmitted through eggshells per wavelength was highest in the UVC range (250–290 nm: 35%–63%), intermediate in the UVB range (290–320 nm: 1.5%–33%), and lowest in the UVA range (320–400 nm: 1%–6%). The absolute irradiance through the eggshell decreased on average across these same wavelengths despite the increase in incident light (fig. 6). Focusing on UVB (since solar UVC barely penetrates the atmosphere and UVA is far less harmful), absolute irradiance across the eggshells ranged, on average, from 0.016  $\mu\text{W}/\text{cm}^2$  (SD 0.003) out of the incident total of 0.047  $\mu\text{W}/\text{cm}^2$  at 290 nm to 0.011  $\mu\text{W}/\text{cm}^2$  (SD 0.003) out of the incident total of 0.758  $\mu\text{W}/\text{cm}^2$  at 320 nm (fig. 6). Although varying by latitude, altitude, time of day, and cloud cover, typical solar irradiance on a clear noon at mean earth-sun distance (1 AU) at the equatorial surface spans from 40 to 80  $\mu\text{W}/\text{cm}^2$  over the same wavelength range (Gueymard 1995). Thus, the bulb we used produced only a fraction of the typical intensity of direct sunlight in the UVB wavelength range, from 0.1% at 290 nm to 1% at 320 nm (see also Ball 1995), rendering any absolute irradiance results in this study conservative and underestimated.

### *P2: IR Light at Natural Intensities Heats the Egg Interior*

This prediction was supported: the interior of the eggs heated when exposed to the IR lamp, and thermocouples

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mittance (T; right column) are shown for two representative village weaver (*Ploceus cucullatus*) eggs of different colors (white and blue-green). To the right of each egg photo, the top row of three graphs displays absolute irradiance values, and the bottom row displays percentages of total incident light. Since R, A, and T are exhaustive as fates of incident light, at any given wavelength across a row of three graphs, the irradiance values sum to the incident light irradiance value (gray box), and the percentage values sum to 100. The effect of color on these dynamics is evident, for instance, in that R and T are higher for the white egg than for the blue-green egg, whereas A is higher for the blue-green egg than for the white egg. Averaging over the wavelengths of 400–650 nm, the white eggshell had R 80%, A 11%, and T 9%, whereas the blue-green eggshell had R 36%, A 56%, and T 7%. Other light sources were used separately to determine the dynamics in the ultraviolet and red-infrared portions of the spectrum (see main text).



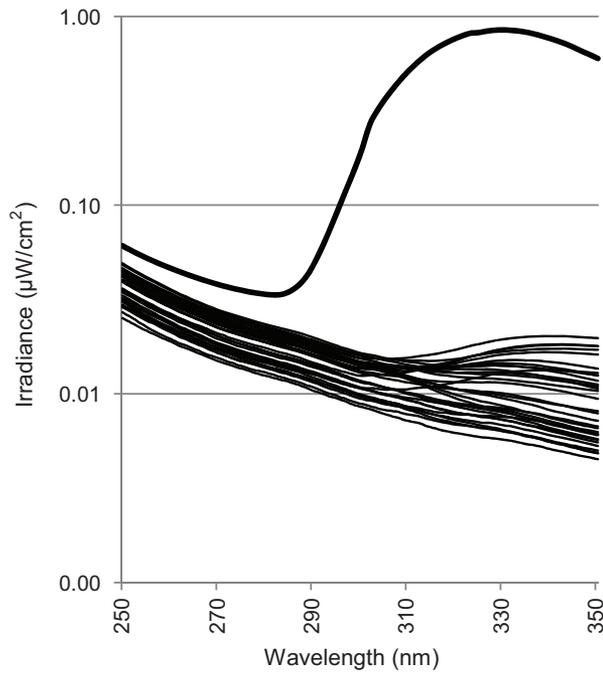
**Figure 5:** Correlation plot relating reflectance, absorbance, and transmittance of light through eggshells in three regions of the light spectrum: UVB (290–320 nm), VIS (400–700 nm), and IR (700–800 nm).

heated more slowly when directly exposed to the lamp than when placed within the eggs at the same distance (mean second-order heating rate constant  $K_2 \pm SE$ : bare thermocouple  $0.121 \pm 0.001$ ,  $N = 13$ ; eggs  $0.0147 \pm 0.003$ ,  $N = 32$ ;  $t_{43} = 5.35$ ,  $P < .001$ ). Incident irradiance from the IR bulb spanned from  $1.56 \mu\text{W}/\text{cm}^2$  at 700 nm to  $4.26 \mu\text{W}/\text{cm}^2$  at 800 nm. By comparison, although again varying by latitude, altitude, time of day, and cloud cover, typical solar irradiance on a clear noon at mean earth-sun distance (1 AU) at the equatorial surface spans from 155 to  $100 \mu\text{W}/\text{cm}^2$  over the same wavelength range (Gueymard 1995). Thus, our

bulb, which heated eggs, produced only a fraction of the typical intensity of direct sunlight in the near-IR wavelength range, from 1% at 700 nm to 4% at 800 nm.

*P3a: Pigment as Parasol: More Intense Egg Coloration Decreases Light Transmittance through Eggshells, including at Damaging UVB and Thermogenic IR Wavelengths*

This prediction was supported: eggs with lower brightness (i.e., with heavier pigmentation) allowed a smaller percentage of light to pass into the interior of the eggshell in the



**Figure 6:** Ultraviolet irradiance through bird eggshells. The upper curve is the absolute irradiance of the light source at the distance that the eggs were situated. The cluster of lower curves represents the irradiance within eggs beneath the light source, representing the light that transmits through the eggshells.

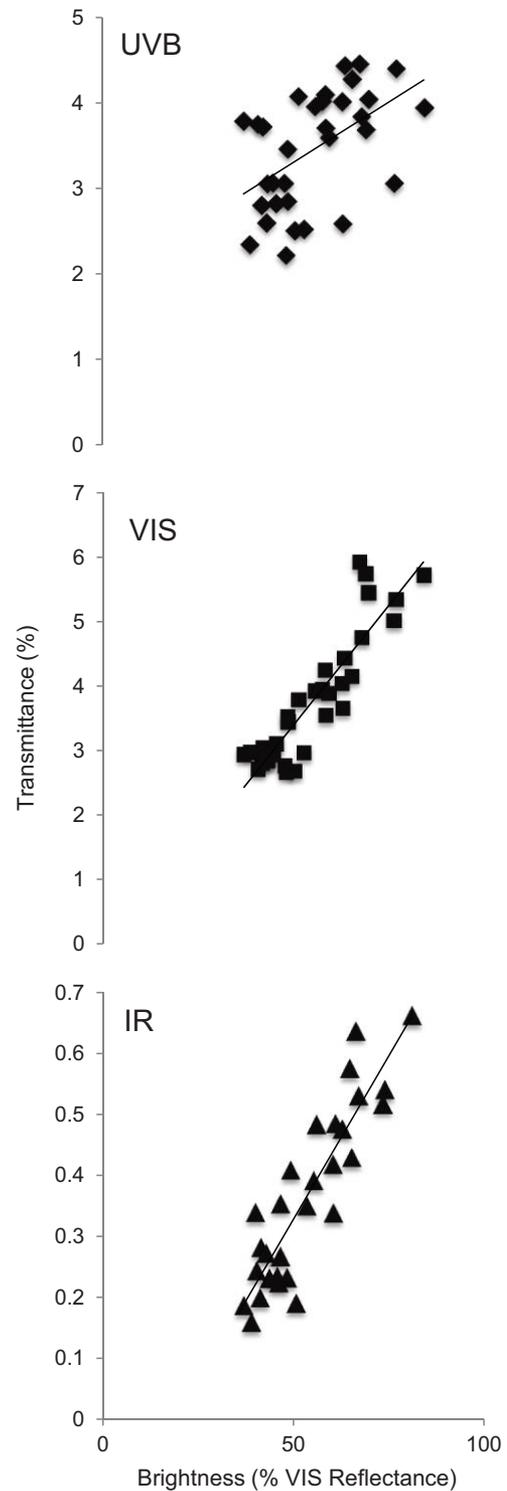
UVB ( $F_{1,30} = 10.82, P = .002, R^2 = 0.27, \beta = 0.52$ ), VIS ( $F_{1,30} = 126.2, P < .0001, R^2 = 0.88, \beta = 0.89$ ), and IR ( $F_{1,27} = 100.3, P = .002, R^2 = 0.79, \beta = 0.89$ ) regions of the spectrum (fig. 7).

*P3b: (If Heating by Transmitted Light Is a Factor)  
Increased Transmittance Increases Heating  
of the Egg Interior*

This prediction was not supported. In fact, transmittance of VIS and IR light was negatively associated with heating rates within the egg; eggs had higher heating rates the less light transmitted across the eggshell in the VIS ( $F_{1,30} = 11.6, P = .001, R^2 = 0.27, \beta = -0.52$ ) and IR ( $F_{1,27} = 28.97, P = .004, R^2 = 0.51, \beta = -0.72$ ) portions of the spectrum; a trend in the same direction was not significant in the UVB ( $F_{1,29} = 3.1, P = .09$ ).

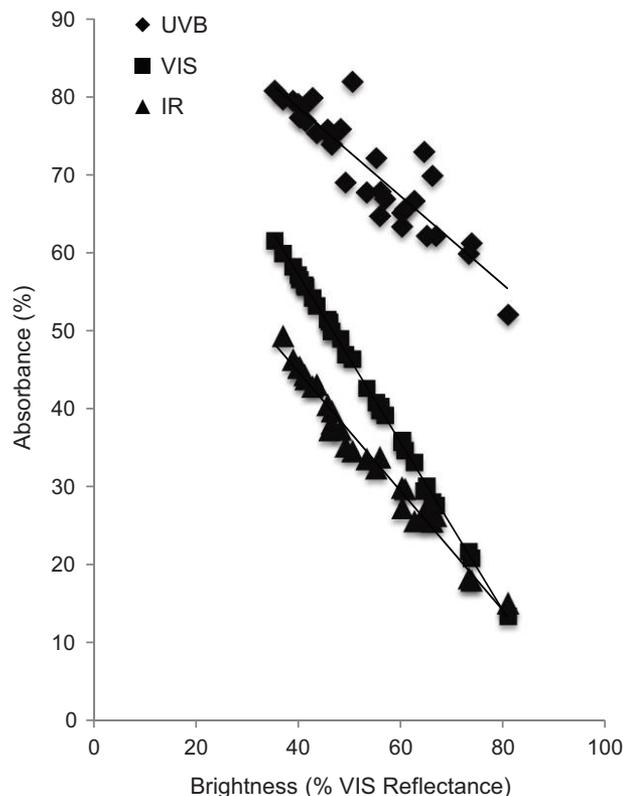
*P4a: Dark Car Effect: More Intense Egg Coloration  
Increases Absorbance of Light by the Eggshell*

This prediction was supported: eggshells with lower brightness absorbed a greater percentage of incident light in the UVB ( $F_{1,30} = 131.6, P < .0001, R^2 = 0.81, \beta = -0.90$ ), VIS ( $F_{1,30} = 2,446.1, P < .0001, R^2 = 0.99, \beta = -0.99$ ),



**Figure 7:** Percentage of light in different regions of the spectrum transmitting through bird eggshells in relation to eggshell brightness.

and IR ( $F_{1,27} = 1,207.0, P < .001, R^2 = 0.97, \beta = -0.99$ ) regions of the spectrum (fig. 8).



**Figure 8:** Percentage of light in different regions of the spectrum absorbed by bird eggshells in relation to eggshell brightness.

*P4b: Dark Car Effect: Increased Absorbance  
Increases Heating of the Egg Interior*

This prediction was supported: thermocouples placed within eggshells of higher absorbance heated at higher rates under an IR lamp (VIS:  $F_{1,30} = 29.16$ ,  $P = .0003$ ,  $R^2 = 0.49$ ,  $\beta = 0.70$ ; IR:  $F_{1,27} = 28.97$ ,  $P = .0006$ ,  $R^2 = 0.52$ ,  $\beta = 0.09$ ; fig. 9). Consequently, if eggs are divided into quartiles by brightness, light-colored eggs (i.e., eggs with brightness in the first quartile) had the lowest absorbance and greatest transmittance in our sample and heated no differently than a bare thermocouple ( $t_{20} = 1.2$ ,  $P = .24$ ). As brightness increased across the sample, eggs heated more slowly ( $F_{1,30} = 29.54$ ,  $P < .0001$ ,  $R^2 = 0.49$ ,  $\beta = -0.70$ ). Patterns over time for a representative white egg and blue egg are presented in figure 10.

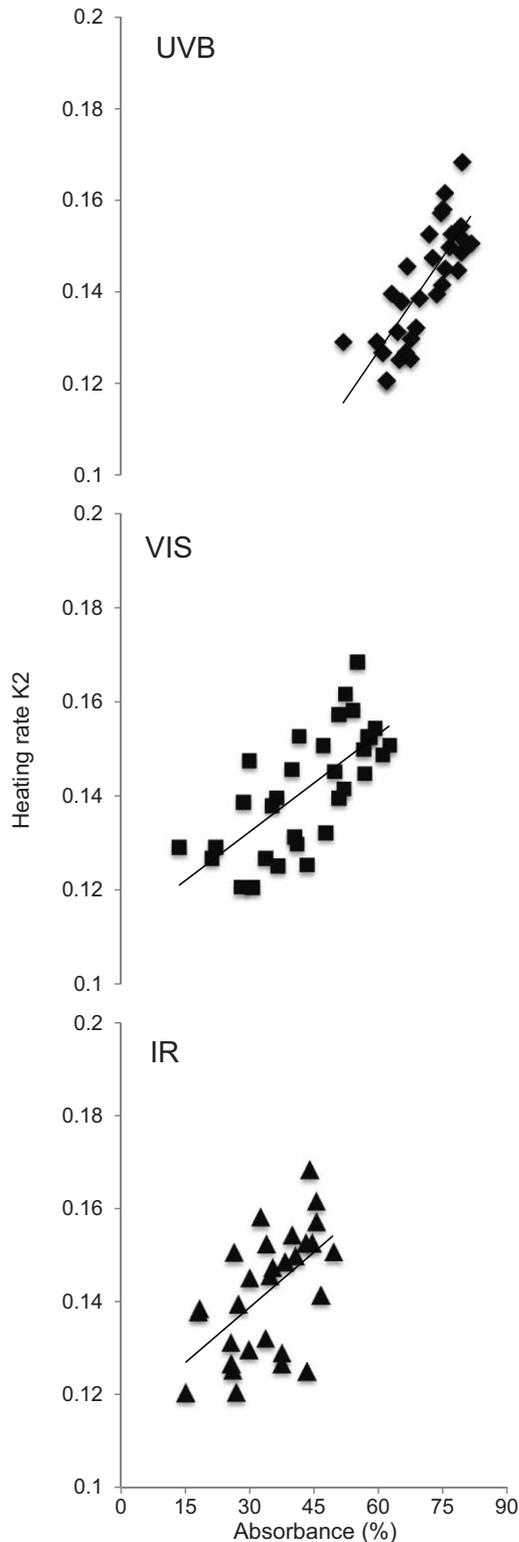
### Discussion

Here we find support for a functional hypothesis for the evolution of eggshell coloration based on solar protection, where pigmentation mediates a trade-off between the dangers of light transmittance and absorbance-related heating. Our study of the biophysical properties of light on eggshells

in a controlled environment validates specific predictions from all four components of our trade-off hypothesis. First, UVB light, even at low intensities relative to natural sunlight, can pass through eggshells; this demonstrates the potential for at least one mechanism by which light transmittance can harm bird embryos (H1 in table 1). Second, the interior of an eggshell heats up when exposed to even low levels of IR light relative to natural sunlight, demonstrating a potential for harmful solar heating of embryos (H2 in table 1). Third, pigmentation acts as a parasol or light shield (H3 in table 1), as more intensely colored eggshells allow less light to transmit, including at damaging UVB wavelengths and thermogenic IR wavelengths. However, transmitted light was not the mechanism for solar heating of the interior of eggshells in our sample. On the contrary, eggshells that permitted high VIS-IR transmittance heated more slowly, likely due to the strong negative correlation we discovered between light absorbed and light transmitted. Fourth, we found support for a dark car effect, such that more intensely colored eggs absorb more incident light and heat up more quickly. When heat gain from absorbance exceeds heat loss to the environment, heat will accumulate on the eggshell and warm the interior of the egg. Bird eggshell color therefore interacts with solar radiation in a complex but biophysically predictable manner, such that more intense coloration could protect the embryo from harmful transmittance, such as in the UV, but at the possible risk of absorbance-related heating, whereas less intense coloration could protect the embryo from overheating at the possible risk of increased harmful transmittance. In some circumstances, the health of the embryo could hang in the balance of this trade-off. White eggs reflect much and absorb little, allowing more light to transmit, compared to more blue-green eggs, which reflect less and absorb more, permitting less transmittance.

Along with feathers and powered flight, one of the most distinctive features in the evolution of the lineage leading to and including birds was the laying of eggs aboveground (Mikhailov 1997). There, eggs could be cared for more meticulously by parents, but they would also be exposed to the environment. They would face predators, physical impacts, and varying temperatures, as well as sunlight. Other animal eggs and embryos exposed to solar radiation often evolve protective traits (Cockell and Blaustein 2001; Blaustein and Belden 2003; Perotti and Diegeuz 2006), so one might expect birds to have done so as well. One might also expect protective mechanisms that have evolved in birds to be unusual among animals, because most bird eggs are not laid in heat-dissipating water as most animal eggs are. Among the several functions of the extraordinary pigments that color bird eggshells, therefore, photoprotection might be one of the more fundamental or original (McAldowie 1886), along with crypsis (Wallace 1889) and perhaps eggshell strengthening (Gosler et al. 2011).

## Features of the Trade-off Model



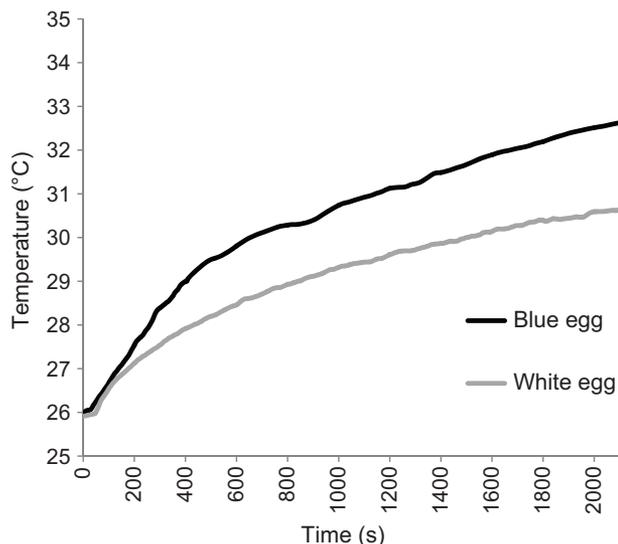
**Figure 9:** Heating rate of the interior of intact eggshells under an infrared lamp in relation to the percentage of incident light absorbed by the eggshell in different regions of the spectrum.

The selective implications of solar radiation for bird eggs and their pigments rely on the particular relationship discovered here between reflectance, transmittance, and absorbance. Reflectance was correlated with transmittance (as in Maurer et al. 2015), and absorbance was inverse to both of them. This set of relationships appears to be the typical case in nature (e.g., in plant leaves; Palmer 1977; Carter et al. 2000; Cabello-Pasini and Macías-Carranza 2011), although other patterns have been observed for some objects, such as some artificial materials (e.g., Chern and Hong 2011).

Our design allowed us to test whether transmittance or absorbance is the more important mechanism by which sunlight would heat embryos. Heating through absorbance of light by the eggshell followed by conduction into the egg is predicted by the dark car effect (P4b in table 1) and is supported by this study. However, heating could also occur through light transmittance through the eggshell and subsequent absorbance and conversion to heat within the egg, including within the embryo itself. These alternatives are analogous to the question of whether sunlight heats a car predominantly through its entry through the windows (and absorbance by the upholstery, for example) or through heating the exterior. For the eggs in our study in the light environment we imposed, the heating trajectory of the egg interior was not positively correlated with transmittance in any area of spectrum but, rather, with absorbance by the eggshell. Still, eggshells of other colors or the light-absorbing contents of eggs (particularly the embryos' dark eyes) might experience transmittance-related heating that could be detrimental to embryonic fitness. Researchers might consider looking for embryonic adaptations to solar radiation—for instance, greater translucence than is usual for vertebrate embryos or protective substances in the eyes.

Any trade-off involves a constraint, but our model indicates that reflectance bears no cost in this context. Why do birds not escape the transmittance-absorbance trade-off by increasing the reflectance of their eggshells? Apparently, eggshells are limited in their reflectance in at least two ways (not including countervailing selection pressures; see below). First, no white pigment exists; such a pigment, with the spectral qualities of polytetrafluoroethylene (PTFE) or barium sulfate paint, for instance, would achieve reflectance higher than calcium carbonate and also prevent transmittance. Second, although bare eggshell has high average reflectance (Fecheyr-Lippens et al. 2015) and thus an increase in its thickness and density would increase opacity and correlate with reflectance to a point (e.g., Burresti et al. 2014), such an increase is presumably limited by the young bird's ability to break the eggshell (Tyler 1969).

Based on the mechanisms we propose for fitness detriment from solar radiation, we predict that the danger of



**Figure 10:** Heating curves of thermocouples placed within two representative intact eggshells of different colors during exposure to a light source whose effective wavelength range extends into the near-infrared. The interior of a blue-green eggshell (black curve) heats more quickly than the interior of a white eggshell (gray curve).

overheating increases much more drastically with more pigmentation than the danger of transmittance increases with less pigmentation—hence the asymmetry of the fitness response curves in figure 1. When a photon is transmitted through the eggshell and into embryonic tissue, it probably has a small additive probability of producing a detrimental effect, such as UV-introduced mutations, which in extreme cases can result in apoptosis of affected cells (Blaustein and Belden 2003; Muñoz et al. 2009). Much of the relationship between photons transmitted and fitness detriment is therefore likely to be linear, or nearly so. When photons are absorbed by the eggshell, on the other hand, they will convert to heat in a wavelength-dependent manner. Heat production above the rate at which heat is lost to the environment will result in heat accumulation and increased eggshell (and in turn embryonic) temperature. The effect on the embryo is systemic rather than cellular, and additional absorbed photons will contribute to the temperature of the embryo. Above healthy embryonic temperatures, the detrimental effect of heating is exponentially related to the increase in temperature in birds, as in other vertebrates whose eggs are exposed to the environment (Carey 2002; Shine et al. 2003). Thus, as exposure to solar radiation increases, selection from both overheating and light transmission increase, but selection from overheating should increase much more steeply. A direct ecological prediction from this asymmetry is that in bright direct sunlight, eggs should be light colored (averaging in an area-weighted fashion across ground color and macu-

lation), despite any dangers of transmittance, to minimize overheating; more colorful eggs are predicted only in more moderate light environments where overheating is less of a danger. Although a broadscale quantitative test has not yet been performed, eggs with light color laid in direct sunlight are common (e.g., those of some ratites, many galliforms, and shorebirds), whereas dark eggs laid in direct sunlight are rare (e.g., loon eggs, which are cooled by water; Walters 1994; Hauber 2014).

#### *Light Environment and Modulating Factors*

In situations where solar radiation is detrimental to embryonic survival, the optimal level of pigmentation in an egg will be driven, at least in part, by the physical effects and trade-off we report here. Thus, to the extent that solar radiation is a major selective factor on the eggs of a particular species, one should be able to predict the intensity of egg coloration based on typical patterns and intensity of light exposure, modulated by other aspects of the environment and of the eggs themselves. The light environment faced by eggs will depend on nest site and structure, the plant community, incubation and other parental behavior, latitude, altitude, and climate. Eggs laid in a forest before leaf-out will be subjected to more light than eggs laid in the same locations afterward. Eggs incubated by both parents more or less constantly or by a female consistently fed by a male might be exposed to little or no light. The danger of solar heating of eggs will depend on ambient temperatures and the extent of air movement, as this facilitates convective cooling. Egg size and eggshell thickness will also be important: smaller eggs can be expected to heat faster with the same density of pigmentation, and transmittance will be greater through thinner eggshells. Other features of eggs might also modulate the effects of solar radiation in ways that are not yet so clear; these include egg shape, eggshell microstructure, fluorescence, gloss, and nonpigment means of egg color, such as chalky bloom applied within the uterus and botanical or fecal stains applied after laying. Overall, in bright light where eggs are in danger of overheating, an egg with less pigmentation can avoid the dark car effect by minimizing absorbance. In more moderate light environments, such as in woodland or grass, where there is still some danger of transmitted light, pigmentation can protect the embryo via the parasol effect. In dark environments, our hypothesis predicts no pigmentation, because the model is restricted to one function for pigmentation: protecting the embryo from light.

#### *Other Sources of Selection*

In nature, even when an egg's light environment is bright enough to be detrimental, the degree to which any predictions deriving from a solar radiation hypothesis will influ-

ence the evolution of egg color will depend on the importance of other selective factors. Ostrich (*Struthio camelus*) eggs, for instance, have been suggested to have the color that balances crypsis and photoprotection (Bertram and Burger 1981; Magige et al. 2008). According to that hypothesis, if they were lighter, they would be more easily located by predators against the darker sand, but if the eggs were darker, they would overheat, as they are exposed to direct sunlight during off-bouts and prior to incubation. For another example, the model species in this study, the village weaver, has a range of colors that has been hypothesized to balance the benefits of brood parasitism detection and photoprotection (Lahti 2008). Some correlative support of this hypothesis derives from the facts that (1) village weaver eggs have evolved variation only in the lighter and not in the darker direction from the apparent ancestral (preparasitism) weaver egg color, consistent with a selective threshold for absorbance-related heating; (2) eggs increase in blue-green chroma (toward that putative ancestral state) after two centuries of release from brood parasitism; and (3) even in populations subject to brood parasitism, the mean egg color in a population with more solar exposure (but still filtered through the greenery of a closed woven nest) is more intensely blue-green than in a population with less solar exposure, consistent with a parasol effect (Lahti 2008). In general, bird egg color can exhibit high lability, and even a single species can exhibit broad heritable variation (e.g., Collias 1993; Lahti 2005; Spottiswoode and Stevens 2012). Thus, dynamically evolving, species-specific balances among selection pressures on color might be common in bird eggs.

One caveat to the results reported here is that the selective effect of solar radiation is not always negative. We have concentrated here on detrimental effects, but not all amounts of heat, and not all intensities of light, are detrimental. Some light exposure in the avian-visible portion of the spectrum actually benefits embryos through multiple effects, including photostimulation, setting circadian rhythms, and antimicrobial defense (Cassey et al. 2011; Cooper et al. 2011; Maurer et al. 2011). Also, moderate egg heating during incubation within a certain range should decrease incubation costs, although the exponential effect of heating makes this a tricky proposition (Barrett 1980; Carey 2002). A thorough model of the impact of solar radiation on bird egg coloration would have to consider these potential advantages along with the dangers covered in this study.

#### *Which Color?*

One important matter to which this study cannot make a significant contribution is how different bird egg hues would vary in the effects described here. As is evident from the stack of curves in figure 3A, the eggs in this study generally had the same overall spectral shape, differing mainly

in average reflectance (brightness) or the height of reflectance peaks relative to troughs (chroma). As brightness and chroma are highly correlated in our sample (fig. 3B), we cannot experimentally separate the relative importance of brightness and chroma to the effects we observed. Also, as we consider eggs that vary on a continuum from blue-green to white, our experimental results do not speak directly to the importance of blue-green as opposed to other colors that bird eggs exhibit. Nevertheless, blue-green is recognized as the most difficult widespread egg color to explain, which is why the quest to explain it has stimulated a burst of recent research (reviewed in Underwood and Sealy 2002; Kilner 2006; Cherry and Gosler 2010; Cassey et al. 2011; Soler et al. 2012; Umbers 2013). Moreover, spectral features suggest blue-green to be the color most associated with the parasol effect. Biliverdin has an absorbance peak in the UV (~380 nm), with a tail extending well into the UVB wavelengths (Kennedy and Vevers 1973; Inomata et al. 2005). Consequently, biliverdin has been hypothesized to function in photoprotection (including of eggs) in invertebrates (McDonagh 2001), fish (Yamaguchi et al. 1976), and lizards (Austin and Jessing 1994). The other pigment in birds' eggs, the reddish-brown protoporphyrin IX, has a similar peak but at a higher wavelength (~410 nm) and has lower absorbance in UVB (Verma et al. 1987), rendering it less effective than biliverdin at blocking UV transmittance into bird eggs. Biliverdin-based bird egg colors also tend to have higher reflectance in the UV than protoporphyrin-based egg colors do (Hanley et al. 2015). These UV-blocking spectral features of biliverdin might be the reason why this odd bile pigment originally spread as a secretion of the avian shell gland.

Certain ecological correlates are also suggestive of a function of blue-green egg color in relation to solar radiation. Lack (1958) noted that blue-green eggs are found most often in moderate light environments, especially in open-nesting forest birds (see also Oniki 1985), consistent with expectations from our model (figs. 1, 2). Our study was originally prompted by Endler's (1993) finding that when the sun is high, ambient light in forests varies from blue to green, depending on the density of the vegetation. Although no quantitative comparison of forest bird egg colors and ambient light has yet been performed, the egg colors of some open-nesting forest birds therefore appear to be broadly coincident with the color of the ambient light. Without function, this situation would be a striking coincidence, especially in light of the restricted range of color space occupied by bird eggs (Hanley et al. 2015). In fact, the presence of blue-green eggs in blue-green light environments might be contrary to expectations based on crypsis: an object is maximally conspicuous if it is highly reflective in the very wavelengths that are predominant in the ambient light spectrum (Endler 1993; Gomez and Théry 2004). Blue-green ambient light might offset this effect by highlighting

the blue-green component of reflectance in surrounding objects as well. Still, blue-green eggs have been shown to be highly conspicuous against the backgrounds of their nests (Siefferman 2006; Hanley 2013). Perhaps these eggs have actually evolved to match the irradiance spectrum of their environment for purposes of photoprotection. For instance, the gray catbird *Dumetella carolinensis*, the American robin *Turdus migratorius*, and the American crow *Corvus brachyrhynchos* all lay blue-green eggs in open woodlands across much of North America. The average nest height in such an environment would correlate with the brightness of the ambient light, with higher nests receiving more light. In these three species, the egg colors follow suit: crows nest highest and have the lightest eggs (in terms of ground color, excluding spots), whereas catbirds tend to nest closest to the ground and have the darkest eggs of the three species. Perhaps crow eggs would tend to heat up in the bright canopy if they were as dark as catbird eggs, and perhaps catbird and robin eggs would unnecessarily allow harmful transmittance if they were as light as crow eggs. Quantitative research must be performed on a variety of species of different egg colors combined with the measurement of light environments to determine whether and why the matching of ambient light contributes to a parasol effect—or, contrary to this, whether it functions as a form of crypsis (Lack 1958) or selectively allows beneficial wavelengths into the egg (Maurer et al. 2011).

With respect to the dark car effect, on the other hand, neither spectral nor ecological considerations yet suggest any generality that biliverdin and protoporphyrin should contribute differently. Both pigments confer high IR reflectance (Bakken et al. 1978), and the density of either pigment should modulate the balance of absorbance and transmittance in the manner demonstrated in this study for largely biliverdin-based egg colors.

#### *Goals for Future Research*

As so little research has sought to test McAldowie's (1886) claim that solar radiation is an ancestral and still pervasive selective influence on egg coloration, the opportunities for investigation remain rich—indeed, largely untapped. Further mechanistic studies could distinguish the importance of different components of color (brightness, hue, and chroma); different portions of the spectrum, including the IR beyond 800 nm; and the influence of ground color versus markings to the effects documented here. We know very little about the way solar radiation interacts with protoporphyrin-based colors, mixed-pigment colors, gloss, and eggshell thickness and microstructure. The roles convection, radiation, conduction, and fluorescence play in the light and heat dynamics of eggs of different colors in sunlight are largely unknown as well. Gas and water exchange might also be components of

this system. All of these tests are best done at first in the laboratory and would yield relative results as in this study. A further range of studies would measure absolute physical effects in actual sunlit environments; although introducing uncontrollable variation, such studies would permit quantification of the actual dangers solar radiation poses to bird eggs in nature. Leaving biophysics for biology, of paramount importance will be tests, in both the laboratory and the field, of hatchability and more specific developmental effects of light and heat on bird embryos. We lack an understanding of the relationship between helpful and harmful solar radiation, the particular effects of UVB on embryos, and more generally, the range of other effects that transmittance might have on embryonic health and survival. Finally, the evolutionary biologist will be especially interested in the outcome of these factors, in terms of natural selection and existing patterns of egg color in birds. However, modulating factors and other sources of selection are probably too pervasive for simplistic broad-brush comparative studies of egg color to be informative. Egg color cannot be predicted based on habitat alone, for instance, and even a knowledge of light environments might not yield effective predictions when nest predation is at all an issue. Careful comparative and experimental studies testing the evolution and functions of egg color in particular taxonomic groups will determine the extent to which the evolutionary history of egg coloration has been influenced by the sun.

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Robin's egg blue: eggs of the American robin (*Turdus migratorius*) from Adams County, Ohio. Blue-green egg color shields bird embryos from sunlight, which can be adaptive as long as light levels are low enough not to heat up the eggs. Photo courtesy of Steve Willson (<http://bluejaybarrens.blogspot.com>).