

Studies of environmental and evolutionary changes in bird egg color

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DEDICATION

To my parents. Thank you for your unconditional love, support including late night (a.m.) pick-ups and homemade arepas every weekend. These things provided me all the fuel I needed to keep going.

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Introduction: A Brief Background of the Study of Bird Eggshell Coloration

Color in the wild has caught the attention of naturalists for centuries. Exploration of foreign lands sparked a curiosity for the creatures that display nature's beauty. For instance, explorer René Lesson described his experience of seeing a bird of paradise for the first time during his expedition to New Guinea in 1824. Holding a gun in his hand, he found that he was not able to shoot the bird because he was in such amazement of its beauty (Jepson 1998). When exercising our human capacities for perception and appreciation, it seems instinctual to be drawn to such colorful and attractive birds. Class Aves displays an incredible diversity of color. Thus, when brave men travelled the world to explore, they naturally wrote of their admiration of nature's exhibition of the color of birds.

Bird egg coloration in particular has been a topic of professional biological study for well over a century. Charles Swynnerton, a naturalist who was particularly interested in aposematism, was drawn to those bird eggs that highly contrasted against their nests with noticeable maculation and bright color (Swynnerton 1916). Swynnerton and other biologists sought to understanding the function and evolution of the array of pigments and forms of spotting they observed. Winson (1921) likewise accompanied his field observations of egg color with possible explanations for the color and markings of bird eggs. He proposed that a bird's age might factor into the paleness or vividness of the egg. Famously, Punnett (1933) sought insights into egg color variation and its heritability by performing breeding experiments with fowl.

The color of the calcareous eggshell is generally divided into two traits, the ground color and speckles (if any). The ground color is the basic and more or less uniform shade of the eggshell, whereas the speckles, often called maculation, are deposited in certain areas of the shell and not others, whether in precise drops or else scrawled or smudged markings. Current research indicates that only two basic pigments are responsible for all bird eggshell pigmentation (Hauber 2014). Both of these are related to the red blood tint hemoglobin (Walters 1994). One pigment is biliverdin IX alpha (***α***), which along with its zinc chelate produces blue and green colors (Kennedy and Vevers 1976). Related closely to bile as its name suggests, biliverdin is responsible for the bluish-white or blue-green ground colors of many bird eggshells (Walters 1994). The second pigment, also heme-related, is protoporphyrin. This generates reddish to brownish hues (Kennedy and Vevers 1976). Protoporphyrin is not only deposited evenly on the shell as ground color, but is also responsible for a diverse array of maculation. When the reddish-brown and blue-green pigments mix, particularly in the ground color, the resulting palette of colors is surprisingly broad, ranging from violet to olive green for instance (Walters 1994; Hauber 2014).

The function or adaption of egg coloration has received significant study. The ancestral state of bird egg color is believed to be white, like the eggs of their reptilian ancestors (Romanoff and Romanoff 1949). Wallace suggested that from the primitively white bird egg, the broad variety of extant eggshell appearances were due to adaptations to their surroundings. This led to his hypothesis of crypsis, where the key function of egg coloration was predator avoidance (Wallace 1889). Over a century later the idea of

crypsis is still one of several current explanations for bird eggshell color. Recognizing the chemical pigments that contribute to the coloration, such as biliverdin, might be a step towards a better understanding of the function of eggshell color in relation to crypsis. McDonagh (2001), for instance, attributes biliverdin's presence throughout the eggshell to the pigment's adaptability, varying from blue to green, based on the cryptic needs of the organism in relation to its surroundings.

An additional problem for open nesting birds is exposure to the elements, including sunlight. McAldowie (1886) suggested that the function of egg color was as protection from harmful solar radiation. In particular, the greenish pigment found in the ground color of eggs might be a photoprotectant (Lahti 2008), as opposed to the reddish tint present in the form of speckles. Sunlight can also cause overheating. Bakken and colleagues (1978) found that biliverdin and protoporphyrin in bird eggshells provide high reflectance in the near-infrared (IR), which in turn minimizes solar heating. They concluded that these pigments provide thermal protection for developing embryos. Little experimental or observational work has tested this hypothesis further, however.

Many species maculate their eggs with what can appear to be a creative splatter of spots. These markings are deposits of mainly protoporphyrin (Kennedy & Vevers 1976). In addition to their role in crypsis, they have been suggested to serve a structural purpose (Gosler et al. 2005). The calcareous eggshell at times may experience and become vulnerable to the thinning of the shell when there is a reduction in calcium. These speckles made up of protoporphyrin may provide reinforcement and strengthen the

eggshell (Gosler et al 2005).

The blue-green pigment biliverdin has antioxidant properties, and might be costly for a bird to produce. When used as a pigment for eggshells, Moreno and Osorno (2003) have suggested that blue-green color serves as a signaling function for females to advertise their genetic quality or condition to males. Consequently, the male might invest in greater paternal care because of the pigment's antioxidant capacity (Moreno & Osorno 2003). Support for this hypothesis is mixed. A comparative study done by Kilner (2006) found no support that blue-green eggs evolved specifically to signal female quality.

A highly recognized breeding strategy such as brood parasitism might play a strong influence on bird egg appearance in some species. The parasite and host interaction can drive a coevolutionary arms race for egg color and speckling (Lahti 2005), as it has with the cuckoos and weaver birds in Africa. Brood parasite egg color can mimic that of its host birds (Hauber 2014), and host egg color might function in recognition of the host's own eggs and discrimination against parasitic eggs (Davies and Brooke 1989). Some birds use both ground color and maculation to distinguish their own from brood parasitic eggs (e.g., Lahti and Lahti 2002).

Looking forward, the functions of bird eggshell coloration are increasingly being explored from the bird's eye view rather than the human visual perspective. As suggested by Cherry and Gosler (2010), an inspection of a combination of elements such as nest surroundings and avian vision were neglected until recently. These are important

modulators of the function of bird egg color and deserve to be investigated.

This thesis contains two studies of bird egg coloration, one focusing on mechanism and proximate change (over the development of an individual embryo), and the other focusing on evolution and ultimate change (over millions of years during the radiation of a diverse group of birds). Chapter 1 tests the influence of light on the color of blue-green bird eggs. A few studies have warned against degradation of egg color over time in museum specimens (Cassey et al. 2010; Cassey et al. 2012), and others have made observations of bird egg color change over the incubation period (Moreno et al. 2011) or in response to particular environmental factors (Avilés et al. 2007; Honza et al. 2012). Here I report a laboratory test of whether ultraviolet (UV), visible light, or infrared (IR) influences the color of blue-green eggs and how this proceeds with time of exposure. Chapter 2 surveys the evolution and function of egg color in the primitive flightless birds known as the ratites (Struthioniformes), as well as the related but extinct elephant-bird *Aepyornis*. Although the eggs of ratites receive popular attention—for instance because of the dark egg of the emu and its tendency to fade, and the large size of ostrich eggs—only the egg of the ostrich has received significant research attention or been described in detail (Bertram and Burger 1981; Hanley et al. 2013).

Attracting attention for their beauty, bird eggs have long been an interest for descriptive biologists and naturalists, but are now important to current researchers searching for insights into the function of egg colors. The following two studies contribute to this current surge of interest in understanding egg color—in particular, what light can do very

quickly, and what evolution can do very slowly, to these precious colors that have been such a notable part of ecological discussion and observation.

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Chapter 1

Light dulls and darkens bird eggs

INTRODUCTION

In 1897, experimental evidence corroborated the impressions of bird egg collectors and museum curators that the colors of bird eggs “are not very fast to light” (Paterson 1897). This initial discovery was thoroughly overshadowed in the subsequent century’s research on egg color, which was dominated by the discoveries that it is consistent within an individual bird and has a strong genetic basis. Punnett’s (1933) breeding experiments demonstrated that egg color differences in Chilean fowl (*Gallus gallus Araucana*) are genetically regulated, and that blue egg color is dominant to non-blue and can combine with gradients of brown through breeding to create a variety of egg colors. Work in this vein has continued to specify the inherited component of egg color (e.g., Punnett and Bailey 1920, Hutt 1949, Francesch et al. 1997, Gosler et al. 2000). Recent research on the genetics of blueness in chicken eggs has culminated in the discovery of a retroviral insertion (*EAV-HP*) that strongly influences this trait (Wang et al. 2013). Concordant with these and other studies of the genetic bases for bird egg color differences, egg color has generally been shown to be robust to environmental variation (Collias 1984, 1993, Gosler et al. 2000, Dearborn et al. 2012, Wheelwright et al. 2012, Duval et al. 2013), licensing claims of evolutionary change from population comparisons (Lahti 2005). Nevertheless, a recent increase in the precision of color measurement (Hill and McGraw 2006) and the proliferation of hypotheses for the functions of bird egg color (Gosler et al. 2005, Moreno et al. 2006, Lahti 2008, Cassey et al. 2011) have accompanied a surge of

interest in the role of environmental and developmental features in modulating or disrupting egg color. This investigation has met with some success: for instance, environmental pollutants have recently been implicated in egg color variation (Jagannath et al. 2008, Hanley and Doucet 2012); and dietary manipulation has elicited changes in egg color (Morales et al. 2011, Butler and McGraw 2013). Moreover, changes in egg color have been observed to relate to bird age (Butcher and Miles 1995), female condition (Siefferman et al. 2006), and laying order (Arendt 2004, Morales et al. 2011). Accumulating instances of small-scale spatial and temporal variation in egg color, especially when correlated with environmental gradients, have also been suggestive (Arendt 2004, Gosler et al. 2005, Avilés et al. 2007, McCormack and Berg 2010, Honza et al. 2012). In all of these cases, the changes in egg color are considered to be modulated by the physiology of the mother, and therefore occur before the egg is laid.

Less researched is the extent to which environmental features directly impact egg color after laying. Some superficial examples of this are well known, such as staining and the wearing off of bloom (Walters 1994). Field researchers typically notice a change in the translucence and perhaps color of eggs after laying, an effect corroborated by the finding of Moreno et al. (2011) that the proportional contribution of blue-green wavelengths to eggshell reflectance declines after laying and through the incubation period in the pied flycatcher (*Ficedula hypoleuca*). Somewhat better investigated is the change in egg color while in museum collections, following analogous work on plumage (Pohland and Mullen 2006, Doucet and Hill 2009). Specifically, the proportional contribution of blue-green wavelengths to eggshell reflectance was lower in museum specimens than in

freshly laid eggs of the song thrush (*Turdus philomelos*), and older egg specimens were less blue-green (according to the same metric) than more recent specimens (Cassey et al. 2010). Moreover, in both this species and in the blackbird (*Turdus merula*), eggs reserved for five years in darkness and measured again were less blue-green as well as lower in reflectance overall (Cassey et al. 2012). Similar results were found in pied flycatcher eggs after 6 months, and again after 23 months, of dark storage (Moreno et al. 2011).

Possible change in egg color with time, particularly in museum specimens, has sometimes been referred to as light-induced fading. There are two problems with this hypothesis. First, the role of light is open to question given the changes that were observed in darkness by Cassey et al. (2012) and Moreno et al. (2011). Second, the only quantitative evidence of egg color change over time is a *decrease*, rather than an increase, in reflectance (Cassey et al. 2010, Moreno et al. 2011, Cassey et al. 2012), which is opposite of the usual meaning of fading as bleaching, i.e., an increase in reflectance, as occurs through photodegradation in a variety of dyed objects such as fabrics (Yoshizumi and Crews 2003), and in coral through a combination of biological and physical mechanisms (Brown 1997). On the other hand, fading could also be taken to mean a loss of chroma (Hanley et al. 2013), i.e., dulling, which is more consistent with past results from eggshells.

Porphyryns, the class of pigments responsible for egg color, are known to photodegrade (Heirwegh and Brown 1982, Moan and Berg 2008). Moreover, some influence of light

on egg color can readily be observed in permanent eggshell displays in bright places; this danger is widely accepted among museum curators, hence the longstanding practice of keeping egg specimens in darkness (Paterson 1897, Cato 1986). Also, in the most spectacular instance of natural change in egg color, emu (*Dromaius novaehollandiae*) eggs can be as dark as the skin of an avocado at an early stage, but will fade nearly to white upon exposure to sun, even over a typical incubation period (Walters 1994).

Here we expose bird eggshells to a known intensity and color of broad-spectrum light in a controlled environment to assess the effect of light on their reflectance. Generalities can best be drawn if experimental eggs vary, but many other things about eggs besides color vary across species (Romanoff and Romanoff 1949). We solved this problem by testing eggs of a species that lays variably colored eggs: Rüppell's weaver (*Ploceus galbula*). Rüppell's weaver egg colors are reported as ranging from a medium blue to white, usually densely spotted with dark red speckles or, less frequently, with tan speckles (Craig 2010). Here we ask whether and how light affects the color of these eggshells, including in what area of the reflectance spectrum changes occur, whether chroma and brightness are both affected, whether initial reflectance matters, and how the effects relate to duration of exposure.

METHODS

Eggs of Rüppell's weaver (*Ploceus galbula*) were collected by DCL less than two days after laying in August 2010 at Awash National Park, Ethiopia (8°53.150' N, 40°02.147' E). Each egg was from a different nest. All eggs appeared to be light to very light blue-

green in ground color, with spots ranging from tan to rust, sometimes so fine or indistinct as to slightly obscure the ground color. Rüppell's weaver is endemic to the Horn of Africa and the southern Arabian peninsula and is not well studied (Craig 2010, Lahti 2013); however, no eggs were taken specifically for the present study. These eggs were collected in order to quantify egg appearance after egg replacement experiments, during which time the weavers promptly renested. On the day of collecting, each eggshell was emptied of its contents and stored in a dark box after drying and until the present study began. Ten eggs were selected for the study based on visual assessment to maximize the range of variation in ground color. See Figure 1 for reflectance spectra of all study eggs. DCL conducted all studies in accordance with permitting regulations in both the U.S.A. and Ethiopia, including permission from the Ethiopian Wildlife Conservation Authority that has jurisdiction over Awash National Park, and according to the recommendations in the Guide for the Care and Use of Laboratory Animals of the National Institutes of Health. The original protocol including the collection and use of eggs was approved by the Committee on the Ethics of Animal Experiments of Queens College, City University of New York (QC IACUC Permit Number 144); no additional IACUC approval was necessary for the subsequent use of eggshells as described in the present study.

The treatment consisted of placing eggs in shallow depressions beneath a light source in a laboratory such that the side of each egg faced towards the light for a total of 108 hours. At specified intervals during this period each egg was removed from the light in order to measure its reflectance: at the start, and then after 4, 12, 20, 28, 36, 60, 84, and 108 hours of light exposure. Reflectance was measured with an Ocean Optics® USB2000 UV-VIS

spectrophotometer, associated Spectrasuite[®] software (Ocean Optics, Dunedin, Florida, USA), a 200 Hz pulsed xenon light source (Ocean Optics PX-2), and a 400 μm reflection probe (Ocean Optics R400-7), held perpendicular to the egg at 7 mm distance, under an opaque cloth. Integration time was set at 80 ms. Reflectance measurements were standardized with a diffuse polytetrafluoroethylene (PTFE) tile that reflects >98% of light over all sampled wavelengths (Ocean Optics WS-1). A rubber stopper was placed over the probe to maintain a consistent angle and distance from the probe to the eggshell. One of us (JYN) made all reflectance measurements, focusing on the center of the eggshell lengthwise, making an effort to avoid spots in order to measure ground color. A photograph was taken of the face of the egg that was exposed to the light, and was consulted in order to ensure that the same area of the egg was measured at every interval, and that the egg was replaced with the same face towards the light after each measurement. Every reflectance measurement was the average of three reads within a 1 cm^2 area of the egg. To detect any change in the spectrophotometer or non-light related changes in egg color over the study period, four additional weaver eggs were measured at each interval but not subjected to the light treatment, remaining instead in a dark box for the duration except when measured for reflectance; no change in the reflectance spectra of these eggs was observed. We gathered reflectance between 250 and 800 nm, a range extending well beyond the bird or human visible wavelengths, allowing us to assess changes in ultraviolet (UV) and infrared (IR) reflectance of the eggshells as a result of light exposure.

We used a 26.67 cm long, 105 watt compact fluorescent bulb (CFS32, US Way

Lighting), which produces 6720 lumens at a color temperature matching that of mean daylight at 6500 K (Judd et al. 1964). By comparison, the color temperature of typical incandescent light bulbs is no higher than 3300 K, and that of ordinary compact fluorescent light bulbs is about 5000 K. We suspended this bulb within a white cone reflector such that the base of the bulb was 29.2 cm above the eggs. This setup resulted in the eggs being exposed to 23.3 W/m^2 of light between 250-800 nm, as determined by absolute irradiance measurements taken with the above spectrophotometer, calibrated to a light source of known power (Ocean Optics DH2000-CAL, deuterium tungsten-halogen), and using a 450 μm optical fiber, with a 3.9 mm PFTE cosine corrector (Ocean Optics CC-3-UV-T). Despite the broad spectrum of the bulb's lighting, 96.1% (22.4 W/m^2) of its power fell (not surprisingly) in the human-visible wavelength range (400-700 nm). Light exposure is compared to selected natural and artificial environments in Table 1, as measured with the same equipment.

We analyzed the change in reflectance over time of light exposure in two different ways: (1) through two steps of statistical data reduction, and (2) through a direct analysis of spectral shape. We used SYSTAT 10 (SPSS, 2000) for all analyses. In the first analysis, we reduced spectral data across wavelengths with principal components analysis and then across time by regression. Ruppell's weaver eggs generally have two reflectance peaks, one in the UVB region (centering around 310 nm) and one blue-green (centering around 500 nm). The unrotated factor loadings were largely interpretable as brightness, blue-green chroma, and UVB-C/IR (Table 2). These three factors explained 78.6, 13.9, and 4.9 percent of the variance, respectively. We calculated the resulting factor scores for

each egg across all reflectance measurements during light exposure. We then ran a linear regression (LR) for each factor per egg, regressing all nine values of the factor against time, in order to reduce the eight temporal transitions to a single slope per egg. We calculated the 95% confidence interval around these ten slopes; if they did not include zero, we rejected the null hypothesis of no change in the factor over time. In the second analysis, we derived three variables directly from the reflectance spectra: the average reflectance across 250-800 nm (brightness); the height of the blue-green peak, calculated as $R_{500} - (R_{400} + R_{650})/2$ (blue-green chroma; Lahti 2008); and the height of the UV peak, calculated as $R_{310} - (R_{270} + R_{350})/2$. The first term in both relations is the typical location of the average peak (maximum); and the second term is the mean of the two typical locations of the troughs (minima) flanking the peak (see Fig. 1). We excluded three eggs from the blue-green analysis because tan spots occluded the ground color and so those eggs did not have the requisite peak. We then conducted a repeated-measures ANOVA across the time of light exposure. For graphical representation we standardized the values of these three variables by subtracting all values during light exposure from the initial value, thus portraying change from the starting condition. Finally, in order to address the question of whether any light-induced change in reflectance differs according to how bright the eggs are, we regressed the total change in brightness against the initial brightness of the eggs. All interpretations are based on $p=0.05$ as the threshold for significance.

RESULTS

All three principal components—interpretable largely as brightness, blue-greenness, and UVB-C/IR respectively—had negative slopes over the period of light exposure, and the associated confidence intervals did not cross zero (Table 2). This effect can likewise be seen by direct inspection of the reflectance spectra and in the changes through time of each egg without exception (see Fig. 2 for two examples). Analyzing all ten eggs together, repeated measures ANOVAs were highly significant across the eight time periods for all three direct spectral variables: incident light caused a decrease on average reflectance ($F_{8, 72} = 11.81, p < 0.000001$), a decrease in the height of the blue-green peak (of the seven blue-green eggs) relative to the surrounding areas of the spectrum ($F_{8, 48} = 11.50, p < 0.000001$), and the height of the UVB peak ($F_{8, 72} = 11.39, p < 0.000001$) (Fig. 3). Eggs with a higher initial reflectance across the spectrum tended to decrease in average reflectance to a greater extent than darker (lower reflectance) eggs (LR: $R^2 = 0.41, p = 0.048$) (Fig. 4). Neither the patterns of residuals from the egg-specific linear regressions, nor the patterns of data points for the individual eggs (Fig. 2) or the aggregated data (Fig. 3), strongly indicate nonlinearity over this duration of exposure, although the initial change in brightness after four hours did tend to be larger than subsequent changes. A rigorous test of linearity would require finer-scaled and evenly spaced intervals of exposure.

DISCUSSION

Exposure to broad-spectrum visible light caused eggshells of the Rüppell's weaver gradually to lose reflectance across the entire measured spectrum of 250-800 nm. Reflectance peaks were disproportionately affected, in this case resulting in a decrease in blue-green chroma and in the smaller UV peak. Lighter eggs were affected slightly more than darker eggs. Analyzing the data by data reduction (PCA) followed by the calculation of regression slopes for each egg produced similar results to the direct analysis of spectral features, with the exception that the change in the factor loading on UV as judged from the PC slope was greater than the change in UV as judged from direct analysis of the spectral peak, likely due to the inclusion of infrared (IR) changes in that PC (see loadings in Table 2). Because PCs can be somewhat less tractable in this way, we find the direct analysis of spectra to be more immediately interpretable when such an analysis is possible. The decrease in reflectance across the spectrum amounts to a slight darkening of eggs following exposure to light. The decreases in the blue-green peak reflectance can be interpreted as dulling, or loss of color intensity. The UV peak extends partly into the bird-visible range, and thus its decrease could be considered partly as dulling as well. These effects also extend into the nonvisible regions of the UV and IR. As with any nearly opaque substance, any radiation that is unreflected will be mostly absorbed, with some smaller proportion transmitted into the egg.

These results are consistent with the qualitative conclusions of Paterson (1897), namely that blue-green eggs became redder and duller following light exposure. He also noted that darker (brownish) eggs did not change as much or at all. The results of the present

study can only be interpreted as relevant to blue-green eggs (i.e., eggs predominantly colored with biliverdin) until similar experiments are performed on eggs of other colors. The decrease in reflectance we observed is also consistent with recent studies of changes in blue-green eggs over time in darkness (Cassey et al. 2010, Moreno et al. 2011, Cassey et al. 2012). Those studies interpreted chroma differently than in this study, as proportional contribution of a certain area of the spectrum to total reflectance, resulting in an automatic change in chroma for any change in brightness. Still, their results do show similar changes in chroma as interpreted in this study as peak height. Remarkably, then, broadly similar changes occur to egg colors over long periods of time in complete darkness, as occur over short periods of time exposed to light. This similarity leads us to speculate that a single process, for instance the oxidative degradation of pigment, might be occurring, some reaction that although not requiring light is hastened by it.

We expect that the changes we observed are caused or catalyzed by photon bombardment of the shell surface, such that additional photons at the same wavelengths will have an additive effect on pigment photodegradation. Our study eggs were reserved in darkness for four years before this study; presumably blue-green eggs tested after a much longer time will still respond to light in a similar way. If this is the case, the results of the present study together with data such as that in Table 1 can be used to generate provisional estimates of the change in spectral reflectance over time of exposure, aside from the rate at which brightness and chroma decrease without light. The experimental lamp in this study resulted in 1% decrease in reflectance of our study eggs in about 30 hours on average, plus 0.5% decrease in the blue-green peak height and a 0.2% decrease

in the UVB peak height over the same time period. If, as indicated in Table 1, fluorescent desk lighting is approximately a third of the power of the study lamp, thus emitting a third of the photons over roughly the same wavelength range, then we might expect one third of the effect. This translates into a 0.011% decrease in reflectance for every hour a blue-green egg in a collection is out of the specimen drawer on a lit table, and an additional half that decrease in blue-green chroma, just from the effect of light. However, this prediction treats ordinary fluorescent light as equivalent to the broad-spectrum, high color temperature bulb used in this study, which might not be the case. Similar rough predictions could be derived regarding the expected decrease in reflectance during incubation in nature, if we knew the dynamics of solar irradiance and duration of incidence on the eggs. Likewise such predictions might be skewed if the sun's UV and IR radiation contributes to the effect on egg color (the study lamp's radiation attenuated rapidly in the UV and IR regions compared to sunlight (Table 1)). More helpful data regarding the effect of solar radiation would come from direct studies of eggshells in sunlight, although variation in sunlight would necessitate regular irradiance measurements throughout the experimental period. Biological relevance would additionally require study of incident light on birds' eggs in natural nests. In addition, previously collected eggshells, refilled, placed into active nests, and measured periodically along with the natural eggs for reflectance changes, could distinguish between changes due to solar radiation and those due to maturation following laying.

Just as different sources of light might have various effects on eggshell color, different species' eggs might not respond the same way, either because of the initial color of the

eggs or because of other eggshell characteristics. The ranges of hue, chroma, and brightness of the eggs in this study were limited, but still variable enough to show that different colors change at different rates. Similar research should be conducted on the eggs of other species, and under other lighting conditions, to determine the importance of this variation. Eventually, since both light and eggshells vary in a limited number of quantitative variables, we should be able to model eggshell reflectance changes in a general way that can accommodate differences among species and light environments. Our results contribute to the growing realization that despite the overall consistency of egg colors across a broad range of environmental and dietary circumstances, some environmental factors do cause egg color to change. We cannot speculate on the possible changes to typical blue-green eggs over incubation specifically due to light because incident light on natural eggs will vary widely due to parental behavior, and nest and habitat characteristics. Still, the fact that direct sunlight is an order of magnitude brighter than our study lamp (Table 2) raises the possibility that the effect of light on blue-green eggs in nature could be important. The implications for museum curation and collections-based research are slight, however: light does dechromatize and darken bird eggshells, at least of certain colors, but it does so gradually. A blue-green egg could be subjected to many hours of study by many researchers in a typical museum light environment before the change in reflectance of a blue-green egg exceeded the measurement error of the most skillful spectrophotometrist.

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Chapter 2

Evolution of egg color in the ratites

INTRODUCTION

Bird eggs are an ideal study subject for an understanding of trait evolution, including responses to natural selection (Walters 1994, Kilner 2006, Hauber 2014). Egg color is thought to diversify under selection for different functions, such as crypsis for predator avoidance (Wallace 1889), distinctive coloration to facilitate egg recognition (Gaston 1993), protection from solar radiation (Lahti 2008), and structural strength (Gosler et al. 2005). Although the ancestral color of all bird eggs is thought to be white (Wallace 1889), the ancient lineage of ratites exhibits a colorful diversity of eggs (Hauber 2014). This chapter provides a preliminary examination of ratite egg color diversity in order to generate hypotheses as to the functions of egg color in these birds. These suggestions are presented in the context of habitat differences and patterns of egg color evolution in a phylogeographic context, given the relationships among species that have been indicated by recent molecular genetic phylogenies.

Phylogeography

The geological breakup of the supercontinent Gondwana set off a series of avian evolutionary diversification events. The most ancient of these divisions, at least in regards to the ancestors of extant birds, was between the paleognaths, which include the ratites and tinamous and are distinguished by their shared basal palate structure, and the

neognaths, which include all other living birds. The paleognaths are considered the most primitive of birds, having begun diverging perhaps in the early Cretaceous period.

The paleognaths are comprised of the flightless ratites, whose sternum lacks the typical avian keel, as well as the flighted (but normally grounded) tinamous. These birds are of great relevance to biogeography and evolution because their history of dispersal and divergence provides insight to the lineage of Aves. The ratites that radiated across the southern hemisphere include the ostrich of Africa, the emu and cassowary of Australasia, the rhea of South America, the extinct moa and the kiwi-- the smallest living ratite--from New Zealand, and the largest of all ratites but now extinct elephant bird from Madagascar. The tinamous populate South America.

The history of the contemporary distribution of the birds in superorder Paleognathae are currently a matter of debate. Not only have the relationships themselves been disputed and revisioned, but the emerging phylogenetic tree creates problems for the classic phylogeographic hypothesis. This hypothesis involves the isolation of lineages on different continents in the order of the separation of land masses during the breakup of the supercontinent Gondwana (Lee et al. 1997). The major movement of these landmasses lasted for approximately 50 million years (during Late Cretaceous and early Paleogene) with the first fragment to break apart being Africa and Madagascar, followed by New Zealand, and subsequently Australia, Antarctica and South America (Ali and Krause 2011). This chronological order yields one hypothesis for the relationships among the ratites and tinamous—the vicariant speciation hypothesis, where contemporary paleognath bird lineages originated when ancestral birds were isolated on

land masses as they split. This hypothesis predicts, for instance, that the sequence of lineage diversification corresponds to the order of land mass fragmentation, that the absolute age of these events also coincide, and that species coexisting on the same land mass today are each others' closest relatives.

The major problem with this idea is the disagreement of the succession of geological fragmentation with the order of the ratite speciation events as it is emerging from recent phylogenetic studies. In the most recent molecular study, which is distinctive for its inclusion of extinct species, Mitchell et al. (2014) found for instance that the Madagascan elephant bird and African ostrich were not each other's relatives, as would have been predicted by the vicariance hypothesis. Rather, the elephant bird was well supported in a sister relationship to the kiwis of New Zealand, a situation which cannot be squared with the geological sequence alone. Furthermore, ratite osteological evidence has been found in numerous fossil localities in Eurasia, compared to very few discoveries in Africa (Mourer-Chauvire et al. 1996). Also, fossil records have shown evidence of a flighted, tinamou-like, palaeognathous lithornithid in the northern hemisphere in the late Paleocene, suggesting a different mode of dispersal (Mayr 2009), not through solely terrestrial means as proposed in the vicariance hypothesis.

With this progress in molecular phylogenetic and paleontological examination of paleognaths, another phylogeographic hypothesis is recently gaining more attention, one that relies on flighted dispersal. This possibility is contrary to the longstanding assumption of terrestrial dispersal associated with the continental breakup. The flighted

dispersal hypothesis also suggests a flighted ancestor of modern ratites, and together with recent phylogenetic evidence challenges the assumption that ratites evolved flightlessness only once. Harshman et al. (2008) produced strong support for the tinamous' position within the ratite lineage rather than sister to it, and on this basis suggested multiple losses of flight among the ratites. The tree of Mitchell et al. (2014) is consistent with Harshman et al. (2008) in this respect, concluding that ratites are not monophyletic and that flightlessness evolved several times in the group. This work is consistent with earlier evidence that kiwis are descended from flying paleognaths (Houde 1986). The currently best supported explanation for the present distribution of ratites and tinamous are that birds flew from land mass to land mass in a manner unpredictable by geography alone, and only later became flightless, separately on different continents. The morphological similarities associated with flightlessness in these lineages are now thought to have resulted from parallel evolution (Harshman et al. 2008, Mitchell et al 2014).

Taxonomic History

Early observations by Merrem (1813) and Beddard (1898) defined a group of large birds that lacked a keel on the sternum, referred to as ratites, meaning “raft-like” for this distinguishing feature. The distinction of ratites from other birds was further described by Huxley (1867), who placed them in their own order. Huxley distinguished ratites by their palates, along with other morphological characteristics. Subsequently the concept of “ratite” was disputed, as some considered the group synonymous with order Struthioniformes whereas others excluded the elephant bird. The tinamous provided

another source of disagreement, either sister to the ratites or in a separate group, often closely allied to the Galliformes (Sharpe 1900).

Nearly over a century later, Cracraft (1981) was the first to apply phylogenetic systematics to the ratites, using morphological character-based analyses. He found a strong link among some species and arranged two suborders, the ratites and tinamous under the order of Palaeognathiformes, a term proposed by Pycraft (1900). Although his positioning of the ratites were very different from what was later described by Cracraft, Pycraft (1900) did include elephant birds under the superorder Palaeognathae. Following classifications based on morphological data (Parker 1895, Cracraft 1981), later studies used various sorts of molecular data such as transferrin immunology, DNA –DNA hybridization, and mitochondrial DNA sequencing (Prager et al. 1976, Sibley and Ahlquist 1990, Cooper et al. 1992).

A recent trio of studies has caused a thorough revisioning of the classic view of ratite taxonomy. Harshman et al. (2008) found tinamous to be nested within the ratites. Haddrath and Baker (2012) concluded that rheas are sister to a group containing kiwis, the emu and the cassowaries; and tinamous are sister to moas. Elephant birds were not included in these first two studies. Mitchell et al (2014), in the most careful analysis to date, which also included ancient DNA from both moas and elephant birds, confirmed the polyphyly of the ratites and concluded that the elephant bird and kiwi are sister taxa, repositioning the remaining paleognaths accordingly.

This study

In this comparative descriptive study, I present the appearance of ratite eggs in phylogenetic context, considering spectrophotometric measurements in the light of literature review and recent proposed relationships among the ratites. I consider the ostrich (*Struthio*) from Africa, the emu (*Dromaius*) from Australia, rheas (*Rhea*) from South America, cassowaries (*Casuarius*) from Australia and New Guinea, and kiwis (*Apteryx*) from New Zealand. I also provide a preliminary discussion of the egg color of the extinct elephant bird (*Aepyornis*) of Madagascar. Thus the moas and tinamous are excluded from this discussion.

METHODS

I measured the color (spectral reflectance) of the ground color of ratite eggs in April 2012 at the Western Foundation of Vertebrate Zoology (WFVZ) in Camarillo, California. I included eggs of all eleven ratite species available in that collection, including eggs from captive as well as wild individuals. Bird eggs at the WFVZ are kept in drawers in a dark, dry and cool location.

Reflectance was measured with an Ocean Optics[®] USB2000 UV-VIS spectrophotometer, associated Spectrasuite[®] software (Ocean Optics, Dunedin, Florida, USA), a 200 Hz pulsed xenon light source (Ocean Optics PX-2), and a 400 μm reflection probe (Ocean Optics R400-7), held perpendicular to the egg at 7 mm distance, under an opaque cloth. Integration time was set at 80 ms. Reflectance measurements were standardized with a

diffuse polytetrafluoroethylene (PFTE) tile that reflects >98% of light over all sampled wavelengths (Ocean Optics WS-1). A rubber stopper was placed over the probe to maintain a consistent angle and distance from the probe to the eggshell. The probe was focused on the center of the eggshell lengthwise, making an effort to avoid spots in order to measure ground color. Every reflectance measurement represented in the results was the average of three reads. I assessed reflectance between 250 and 800 nm, a range extending well beyond the bird or human visible spectrum in both the ultraviolet (UV) and infrared (IR) directions. Graphs of raw spectra and sample egg photographs are presented.

RESULTS AND DISCUSSION

Ostriches

The ostrich lives in the sub-Saharan region of Africa and is the largest extant bird (Campbell and Lack 1985). When Gondwana began to split in the Cretaceous, Africa and Madagascar were the first landmasses to separate, approximately 130-100 Mya (Ali and Krause 2011). According to most molecular studies, and consistent with the vicariant speciation model, the ostrich holds the basal position of the ratite (Harshman et al. 2008) and paleognath phylogeny (Haddrath and Baker 2012; Smith et al. 2012; Mitchell et al. 2014).

The eggs of the ostrich are large (mean 17.8 x 14 cm) (Hauber 2014), immaculate, and have been described as off-white to cream in color (Hauber 2014) with a glossy sheen

(Tyler 1964). I measured eggs from 5 individuals including one full 22-egg clutch of the Massai Ostrich (*Struthio camelus massaicus*) (Fig. 3), and also single representative eggs of other individuals of both extant species, the Somali ostrich (*Struthio molybdophanes*) and the common ostrich (*Struthio camelus*) of subspecies *S.c. camelus*, the north African ostrich and *S.c. australis*, the southern ostrich. Substantial variation in overall reflectance is evident between species and among subspecies (Fig. 2). The 80 year older wild egg of the North African ostrich (*Struthio c. camelus*) was brighter than the more recent egg of a captive bird, which could simply represent natural variation, or else an effect of age since laying. The sample eggs of the Massai ostrich (*S.c. massaicus*) and the captive north African ostrich (*S.c. camelus*) not only show minimal reflectance differences along the spectrum but also share a similar and distinct small UV peak that is not evident in the other three taxa.

Ostriches nest in the African savannah and desert. The nest is an exposed, communal, shallow pit in the ground with a normal clutch size of about 20 eggs (Hauber 2014). The brightly colored, conspicuous eggs laid in a shallow and open pit could attract predators, but if they were darker they might become overheated. Moreover, the nest is normally left unattended during the first week of the laying period, the eggs being left uncovered (Bertram and Burger 1981). Perhaps the thick (2 mm) shell of the eggs (Campbell and Lack 1985), in combination with some pigment, might filter harmful solar radiation. On the other hand, its top predator, the Egyptian Vulture (*Neophron percnopterus*), hunts visually and would notice brightly colored eggs against the darker sand. Thus the color

of the ostrich egg—lighter than would be predicted for crypsis but darker than would be predicted for filtering sunlight—might reflect a tradeoff between predation avoidance and protection from solar radiation (Bertram and Burger 1981, Magige et al. 2008). If the eggs were darker they would be more cryptic but could risk embryonic overheating. Perhaps to ameliorate this tradeoff, these birds have evolved a behavioral response as well: they are extremely efficient and powerful in defending their nests. Thus, a cream to near-white eggshell in this species, although conspicuous to predators, might be retained by a combination of the benefit of avoiding overheating (Bertram and Burger 1981) and the behavioral ability to protect their nests from predators (Kilner 2006).

Rheas

The two species of rhea (*Rhea*) are native to the eastern subtropical and temperate grasslands of South America (Campbell and Lack 1985, Hauber 2014). In past phylogenetic studies, the rhea has been clustered with extant Australasian ratites such as the cassowary and emu (van Tuinen et al. 1998, Sibley and Ahlquist 1990), and the kiwi. More recent molecular phylogenies have been inconsistent in the placement of the rhea (e.g., Harshman et al. 2008). With the inclusion of ancient DNA, Mitchell et al. (2014) proposes that it is basal to all the paleognaths (including the extinct moas and elephant bird) besides the ostrich. South America was one of the last landmasses to separate from Gondwana, about 30- 50 million years ago. Ratite fossil evidence dated from the Paleocene (about 56 Mya) found in South America includes a possible relative of the rhea, *Deogenornis*, which resembles extant birds (Mayr 2009).

Rhea eggs are about 13 x 8.9 cm in size (Hauber 2014). Their eggshells are immaculate and have been described as glossy as ostrich eggs (Tyler 1964), colored light greenish to yellowish (De La Peña 1999) to bluish yellow. As with the more dramatic changes in emu eggs (see below), rhea egg ground color fades to a dull cream when exposed to sunlight (Hauber 2014). I measured eggs of both extant species, seven individuals of the greater or American rhea (*Rhea americana*) and thirteen individuals of the lesser or Darwin's rhea (*Rhea pennata*), including two subspecies *Rhea p. pennata* (12 individuals measured) and *Rhea p. tarapacensis* (one individual measured). I measured two full clutches, one of nine eggs and another of 20 eggs, both of *Rhea p. pennata* (Fig. 4). The sample eggs vary in blue-green and UV chroma, some *Rhea p. pennata* individuals exhibiting more noticeable peaks than others, with the least chromatic being that of *R. p. tarapacensis*. Comparatively there was little variation in brightness among the eggs. All eggs were collected about the same time, a century ago.

The polygamous rheas lay their eggs in a shallow ground scrape lined with dry vegetation or branches and sticks, often near bushes (Campbell and Lack 1985). They are communal nesters, with about 5-10 eggs laid per female, and an average of 26 eggs total per nest (Hauber 2014). The male parent is protective of his nest, from predators or other rheas (Campbell and Lack 1985). Some slight crypsis coloring, in response to predators (Kilner 2006) might explain the cream-toned egg with underlying blue/green to yellow. Rhea eggs also experience significant exposure to the sun; although some sunlight may contribute to healthy embryo development, too much pigmentation in sunlight can

destroy the embryo (Bertram and Burger 1981). It is probable that a small amount of blue green pigment contributes to protect the egg but also regulates the amount of sun exposure (McAldowie 1886). Assuming that the ancestral egg to all paleognaths was white, and that no blue-green is evident in ostrich eggs, the slight amount of blue-green chroma detected in our results and through previous qualitative visual observations suggests that this represents a notable egg color change in the ratite lineage.

Cassowaries and emus

Australia divided from Gondwana approximately the same time as South America, 30 to 50 million years ago. Both the cassowary (*Casuarius*) and emu (*Dromaius*) are native to Australia, with the cassowary range extending into New Guinea. The cassowary and the emu have been consistently supported as sister taxa in morphological and molecular analyses (Cracraft 1974, Sibley and Ahlquist 1990, Haddrath and Baker 2001, Zelenitsky and Modesto 2003), and have often been considered an order to themselves (Casuariiformes). Several osteological characters are distinctive to these two lineages (Cracraft 1974). Their relationship is consistent with the continental vicariance model, and they are believed to have split from their common ancestor with the elephant birds and kiwi about 27 mya (Mitchell et al. 2014).

Although they live in different habitats—the cassowary in tropical forests and emu in savannah woodlands and semi arid grasslands—the sister taxa have similar diets and breeding biology (Campbell and Lack 1985). The egg texture of both species are similar,

with rough grainy grooves and an overall dominant green color, with cassowary eggs being less rough (Marchant and Higgins 1990). The degree of greenness is the most notable difference between the eggs of the two genera.

Cassowaries

The omnivorous cassowary is found in the lowland rainforests of Australia and New Guinea, as well as nearby Indonesian islands (Hauber 2014). It lays a clutch of 3-5 eggs of about 14 x 9.5 cm in size (Hauber 2014). Cassowary eggs have been described as having a background of pale green (Hauber 2014), or light pea-green (Marchant and Higgins 1990), to a darker pea green, perhaps in fresher specimens (Campbell and Lack 1985). The distinct grainy texture that covers the egg appears darker than the background color (personal observation).

I measured 11 representative eggs of three extant species: the southern or double-wattled cassowary (*Casuarius casuarius*), the dwarf or Bennett's cassowary (*Casuarius bennetti*), and the northern or single-wattled cassowary (*Casuarius uniappendiculatus*). I also measured two 4-egg clutches of *C. casuarius*. I found little difference between wild and captive species or within a clutch, although chroma was variable among individuals (Fig. 5). Spectral shapes illustrate the green chroma and yellowishness in the background that are characteristic of cassowary eggs, whether captive or wild. The one *C.uniappendiculatus* specimen measured was significantly brighter than the rest.

Cassowaries lay their eggs in a large ground nest built from leaves and other plant

materials (Hauber 2014). Considering this nest site and the tropical rainforest environment, possibly the color of their eggs functions in crypsis (Wallace 1889) from mammalian predators, as the greenness would soften the reflected light in a forest (McAldowie 1886).

Emus

The Australian emu is an omnivorous bird that lays its eggs in a shallow depression in the ground lined with plant matter (Eastman 1969, Hauber 2014), in clutches of 11-20 eggs (Hauber 2014). A single nest may contain eggs from multiple fathers and mothers. The average egg is slightly larger than those of the cassowary at 15.2 x 8.9 cm (Hauber 2014). Similar to the cassowary egg, emu eggs are not smooth, but have textured grooves. These are much more pronounced in the emu, and their dark green contrasts greatly with the lighter blue-green background color (personal observation). Emu eggs are opaque and thick shelled (Kjelland et al. 2012), dark olive-green (Marchant and Higgins 1990), and are often described as being rough textured and the color of an avocado (Hauber 2014), even at times darkening to an indigo or almost black. When the eggs are first laid, the egg is light green, darkening to a black-green during incubation (Eastman 1969). Emu eggs have a tendency to fade in sunlight if left unattended (Marchant and Higgins 1990, Hauber 2014), which they almost never are.

I measured eggs of 17 individuals of the emu (*Dromaius novaehollandiae novaehollandiae*), including one eight-egg clutch. I also measured one single egg and

one nine-egg clutch of a proposed but disputed second species, the spotted emu (*Dromaius irroratus*). The emu eggs I measured showed little variation, with reflectance being very low (dark) in all eggs except for two, which displayed noticeable fading or bleaching (increase in reflectance). There was a small blue-green chroma peak in *D. novaehollandiae*, which was even smaller in the occasionally proposed separate species *D. irroratus* (Fig. 6). The blue-green peak in *D. novaehollandiae* may be more evident because several of the eggs measured were collected recently and were laid by captive birds.

Emu nests are shallow depressions on the ground lined with plant materials (Eastman 1969, Hauber 2014), where the male incubates for long periods of time, forfeiting foraging while incubating (Campbell and Lack 1985). This strategy allows the eggs to be protected from predators (Eastman 1969) and from overheating (Bertram and Burger 1981). Apparently there are no hypotheses as to why these eggs have evolved to be so dark, which is especially striking since dark eggs are so rare (Maurer et al. 2011). Whatever the reason the dark egg color evolved, evidently the overprotective incubating male effectively protects the eggs, enabling the survival of emu eggs despite their color (Swynnerton 1916). The spectral difference between cassowary and emu eggs is obvious to the human eye, with cassowary eggs exhibiting blue-green chroma peaks with some yellow, as opposed to the emu eggs' very low level of brightness with only a small green peak.

Kiwis and elephant birds

Based on the latest phylogeny of ratites (Mitchell et al. 2014), the chicken sized nocturnal kiwis from New Zealand and the large elephant birds from Madagascar are sister taxa. Thus two New Zealand lineages — the kiwis and the extinct moas — originated separately following the split of New Zealand from Australia in the late Cretaceous. In the past these two lineages were considered sister taxa (Parker 1895). The findings of Mitchell et al. 2014 therefore not only rearrange the ratite phylogeny but raise questions as to the timing of ratite radiation and dispersal. The sister relationship between the kiwis and elephant birds on opposite sides of the world conflicts with the timing of the breakup of Gondwana, and suggests flight as a form of dispersal. These lineages appear to have diverged about 50 mya, when the last few landmasses were fragmenting (Mitchell et al. 2014). Despite the stark differences in size between the two groups of birds, there are some morphological similarities (Mitchell et al. 2014). Although their egg sizes are incomparable because of the great difference in body size, it is notable that elephant bird eggs are the largest ever discovered, and that kiwi eggs are the largest eggs in relation to their body size (Hauber 2014).

Kiwis

The shy nocturnal kiwis (*Apteryx*) inhabit the forest understory of New Zealand (Campbell and Lack 1985, Hauber 2014). Their one to two egg clutch is laid in an underground burrow below vegetation (Hauber 2014). Each egg is about 18-25 percent of the female's body weight (Campbell and Lack 1985), and averages 12 x 7.9 cm in size

(Hauber 2014), being the largest egg in relation to adult body size of any bird in the world. The yolk is also 50 to 100 percent larger in relation to its egg size than that of other birds (Andrews and Harris-Ching 1991). Their egg color has been described as white to beige with a bluish tint (Hauber 2014), or immaculate greenish-white (Marchant and Higgins 1990).

Only three eggs were measured: two of the North Island brown kiwi (*Apteryx mantelli*) and one of the Southern brown kiwi or southern tokoeka (*Apteryx australis*). Among the kiwi eggs I measured, a small blue-green peak was evident in the captive *A. mantelli* but not in the two wild specimens, possibly due to degradation of blue-green chroma over time in the latter, as they were both about a century older. Based on the few eggs measured, reflectance measurements for both *A. australis* and *A. mantelli* appear similar even though they were collected 100 years apart. Interpretation is compromised by the small sample size, however.

In darker situations, eggs tend to be white, as most functions of pigment might relate to visibility (McAldowie 1886, Lack 1958). Considering the partly underground nest of the kiwi and consequentially the minimal sunlight exposure, the whitish egg color and the relative thin shell might help to maximize solar exposure for embryonic development (McAldowie 1886). Perhaps the slight blue-green peak evident in our results helps protect the very thin eggs from excess solar radiation.

Elephant birds

Although not much is known about the elephant birds (*Aepyornis* and *Mullerornis*), they left behind several eggs for scientists to analyze. These enormous herbivores were apparently populous on Madagascar (Fuller 1987) at one time, but became extinct in the 1600s -1700s, presumably because of humans. Their fossil history is restricted to the Pleistocene, but recent molecular evidence supports the divergence of the two known genera, *Aepyornis* and *Mullerornis*, approximately 17 mya (Mitchell et al. 2014). Fossil evidence has been most forthcoming from presently isolated areas, highlighting the possibility of human encroachment. Their eggs, very large at 34.3 x 24.1 cm (Hauber 2014), have been considered prized possessions, and have been discovered buried in the sand of beaches, dunes, and riverbanks (Hauber 2014). The French governor of Madagascar described in 1658 a large bird that “lays eggs like the ostriches; so that the people of these places may not take it” (Fuller 1987). The egg color has been described as ranging from an off-white to peach (Hauber 2014). Their breeding habitat was probably wet forests (Hauber 2014). Their incubation habits and clutch size are unknown.

Ancestral elephant birds (from about 50 mya) appear to have inhabited an island environment with few or no predators (Mitchell et al. 2014), allowing these birds to nest openly (like ostriches), without danger of depredation. Perhaps their ancestral egg color was whiter than more recent eggs, and crypsis became a selective pressure only later with the advent of predation on Madagascar. The beige color of recent eggs possibly suggests crypsis in a time period of increasing depredation (Wallace 1889).

Measurements of the extinct elephant bird (*Aepyornis maximus*) egg were taken at multiple locations of each of three eggs, including one still unbroken and containing embryonic remains. There was little variation within an egg, but overall reflectance was higher (brighter) in the two eggs without contents than with content, and the eggs differed in chromatic variation to some extent, including in the UV (Fig. 8).

CONCLUSIONS

From a presumably white ancestral egg color (Wallace 1886), our most primitive extant birds, the paleognaths, have evolved an impressive diversity of egg colors, from the phenomenally glossy colors of tinamous to the matte off-white of kiwis. We might gain an understanding of how this diversity arose by looking at paleognath egg colors in a phylogenetic context. Apparently the paleognaths, including both the ratites and tinamous, diversified through a combination of vicariance following continental drift during the Gondwana breakup (Lee et al. 1997, Cracraft 1974), and, highlighted in recent phylogenetic studies, through flighted dispersal (Harshman et al. 2008, Mitchell et al. 2014). This preliminary comparative study provides one perspective of the evolutionary history of ratite egg colors in the light of our current understanding of their phylogenetic relationships and phylogeography. Three tentative conclusions follow from the above descriptions.

First, although the ratite genera diverged millions of years ago and are broadly dispersed geographically, my observations indicate that relatively closely related species tend to have a similar color to each other (Lack 1958). This is uniformly the case between

species in the same genus, such as the species of *Struthio*, *Apteryx*, and *Rhea*. The most significant exception here is evidently *Casuarius*, where one species (*C. uniappendiculatus*, at least in the one specimen I measured) is much brighter and less chromatic than the other two. Similarity is also evident between sister genera. For instance, the greenest eggs among the ratites are the dark green emu eggs and the cassowary's paler green eggs; moreover, both have raised pigment-dense ridges, adding texture to the eggs. The kiwi-elephant bird clade displays more neutral eggshell colors, with the kiwis having white to a beige eggs with a bluish tint, and the extinct elephant bird ranging from off-white to peach (Hauber 2014).

Second, the pigments responsible for bird eggshell color have evidently been deeply conserved throughout these ancient radiations. Protoporphyrin is the pigment that is responsible for browns in eggshells, and biliverdin is responsible for blue-green (Kennedy and Vevers 1976). Traces of these tetrapyrrole pigments have been found preserved in separate species of extinct moa eggshells (Ilgic et al. 2010), and both biopigments are widely found in bird eggshells today (Mikšić et al. 1996). The diversity of colors indicated here for the ratites require highly varying degrees of concentration, resulting in colors from off-white to indigo black; nevertheless just these two pigments appear to be sufficient to explain this broad diversity and have remained so over tens of millions of years.

Third, ratite egg colors appear to have resulted from a variety of selective pressures. No one function is sufficient to explain the diversity of ratite egg appearance (Cassey et al.

2010), attesting to egg color as a model system for trade-offs among different selective pressures (Kilner 2006). The simplicity of the off-white ostrich egg and the pale greenish to bluish yellow rhea egg, both moderately glossed, together with a lack of close relationship, suggests similar selection pressures in the open communal nests they both have. In fact, the grassy substrate in South America as compared to the sandier terrain in the ostrich's range seems to accord well with the differences in egg color in the two genera. Apparently the same selection pressures that are important for these ancient birds are of the same general form as more derived birds experience. One possible exception is the emu, whose extremely dark eggs are currently without evolutionary explanation. In fact, the peculiar texture of both emu and cassowary eggs requires functional investigation. Future work should also take into account avian vision (Hanley et al. 2013), as the way the birds themselves see their eggs could illuminate the relevance of certain competing hypotheses.

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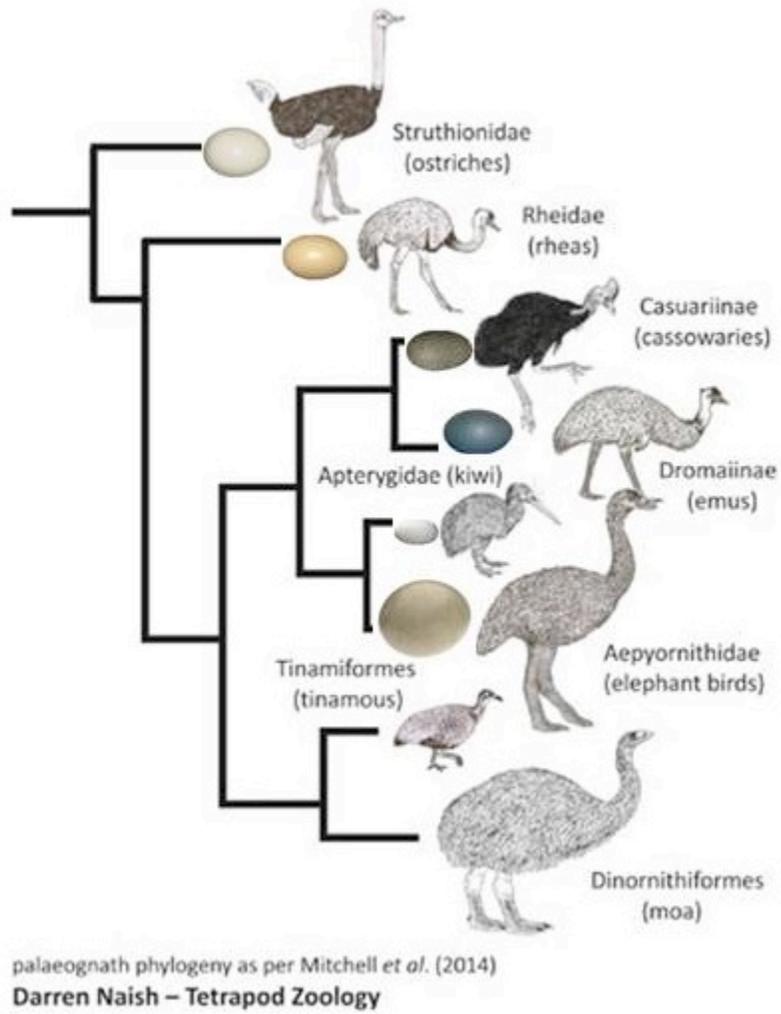


Figure 1. Ratite phylogeny with sample egg images.

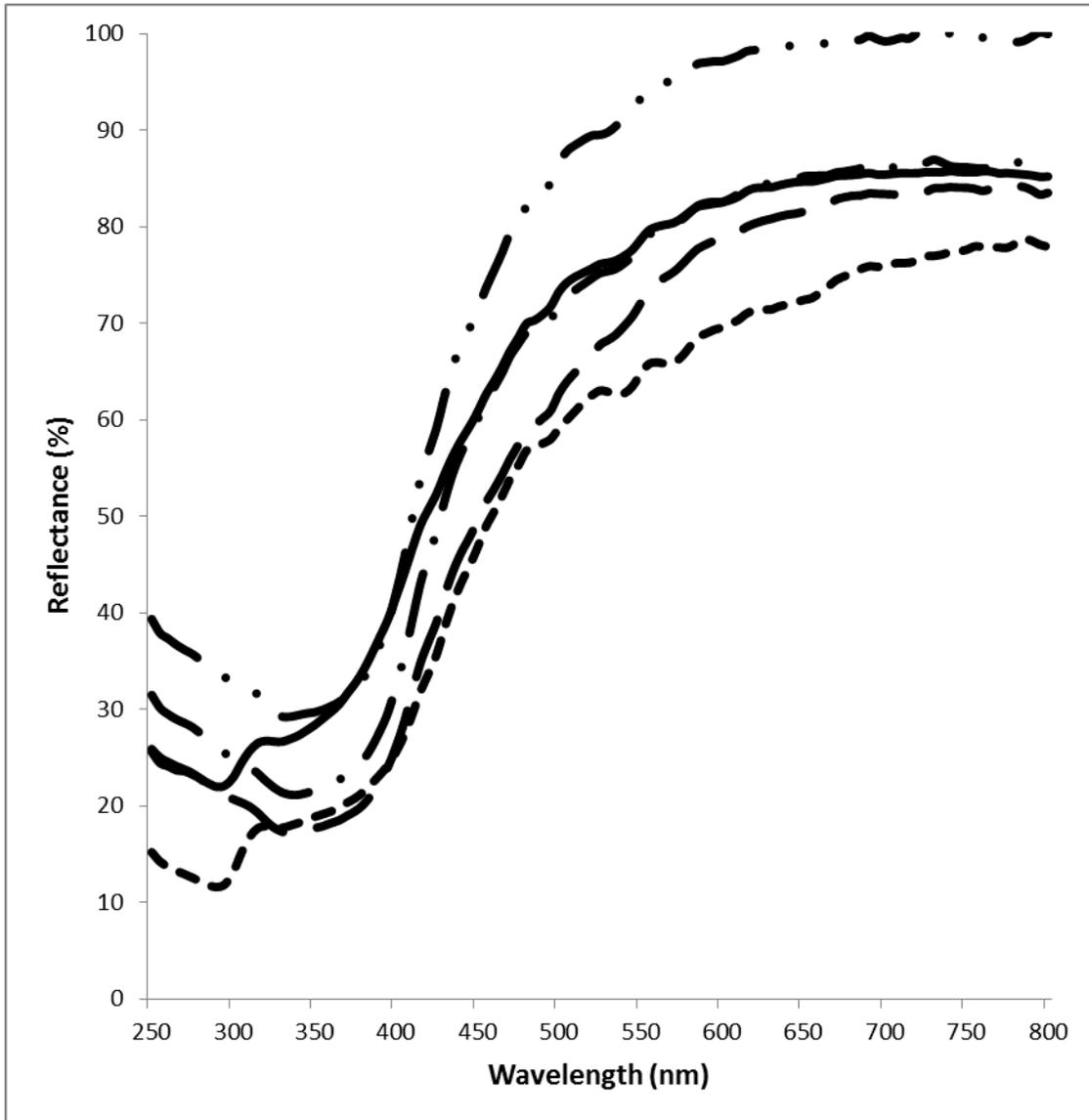


Figure 2. Ostrich egg spectra, variation between species and among subspecies. Both extant species and three subspecies are shown: the Somali ostrich *Struthio molybdophanes* (dashed and double dotted line) and the common ostrich *Struthio camelus*, of the following subspecies: Massai ostrich *S. c. massaicus* (solid line), north African ostrich *S.c.camelus* (wild: dashed and dotted line; and captive: dotted line), and the southern ostrich *S.c.australis* (dashed line). Each line represents one egg of a distinct individual with the exception of *S.c.massaicus*, where the line represents the average a twenty-two egg clutch (see Fig.3).

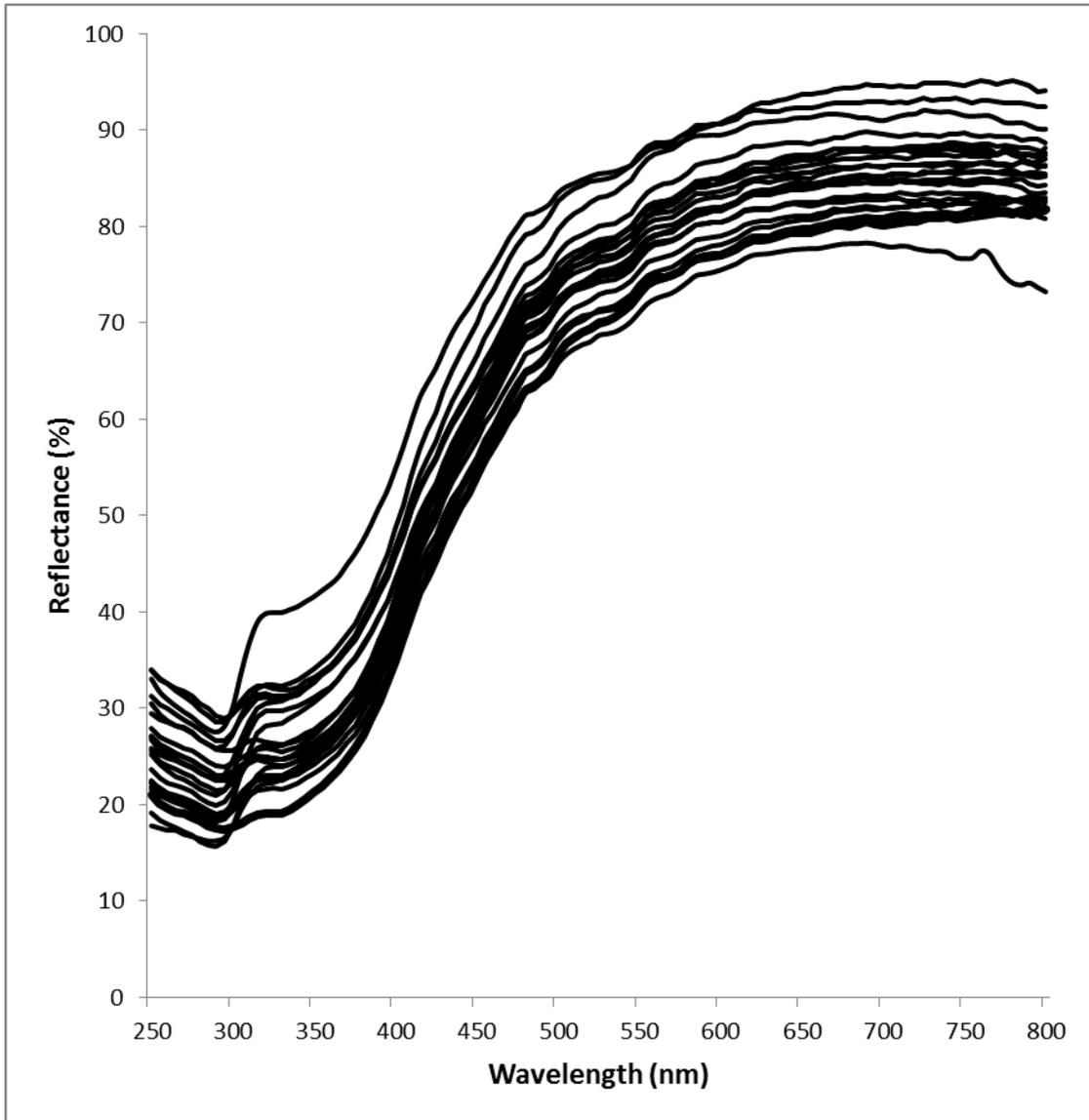


Figure 3. Massai ostrich (*Struthio camelus massaicus*) egg spectra, within-clutch variation. A single clutch with twenty-two eggs.

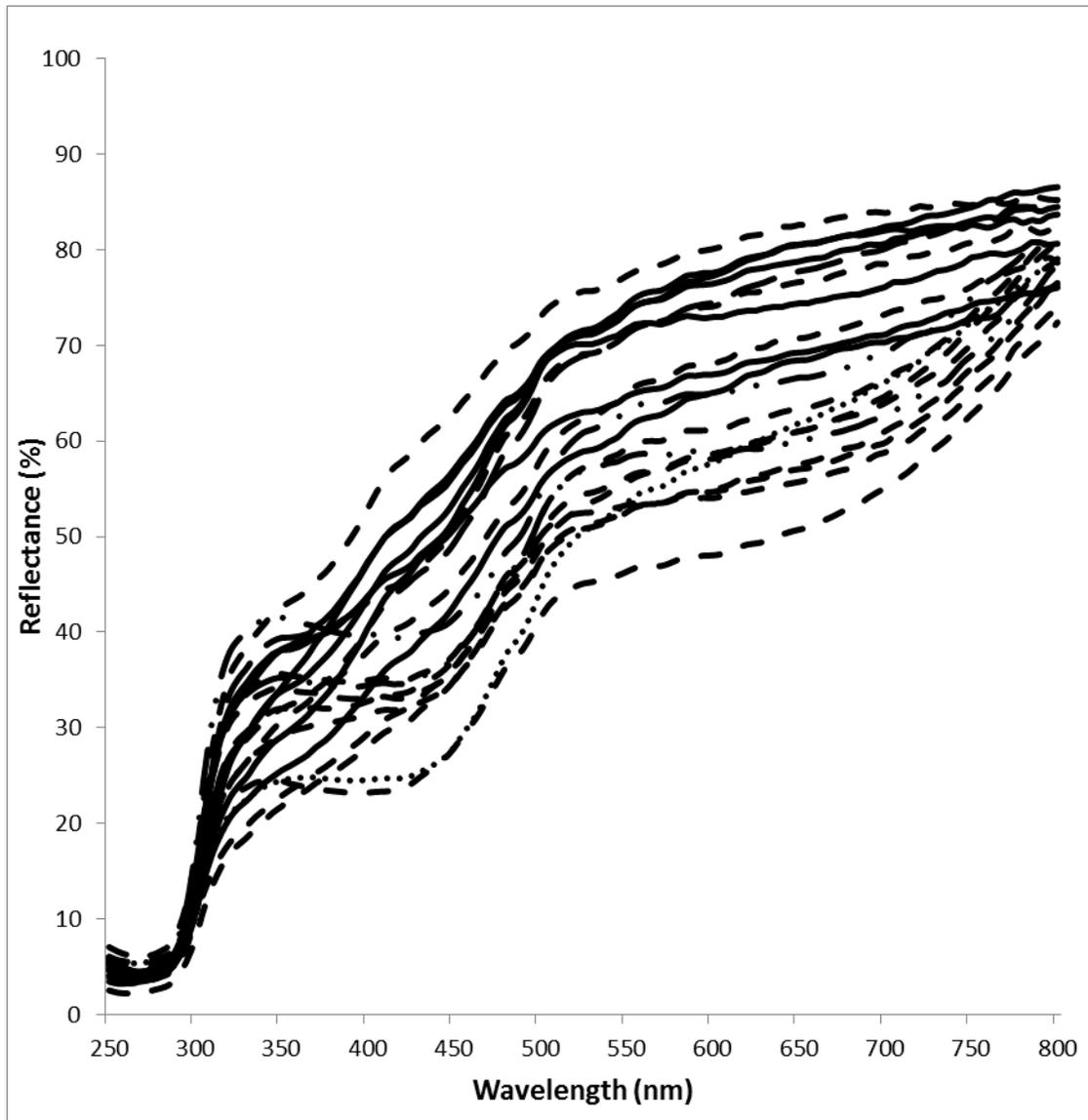


Figure 4. Rhea egg spectra, variation between species. Both extant species are shown: the greater or American rhea *Rhea Americana* (six solid lines -wild, one long dash line -captive), and the lesser or Darwin's rhea *Rhea pennata* of two subspecies: *Rhea pennata pennata* (ten dashed lines -wild, two long dash dot dot lines - captive) and *Rhea pennata tarapacensis* (one round dotted line). Each line represents an average of individuals within each species (including an average of a nine-egg and a twenty-egg clutch from wild *Rhea pennata pennata*) with the exception of *P. tarapacensis*, which represents a single egg.

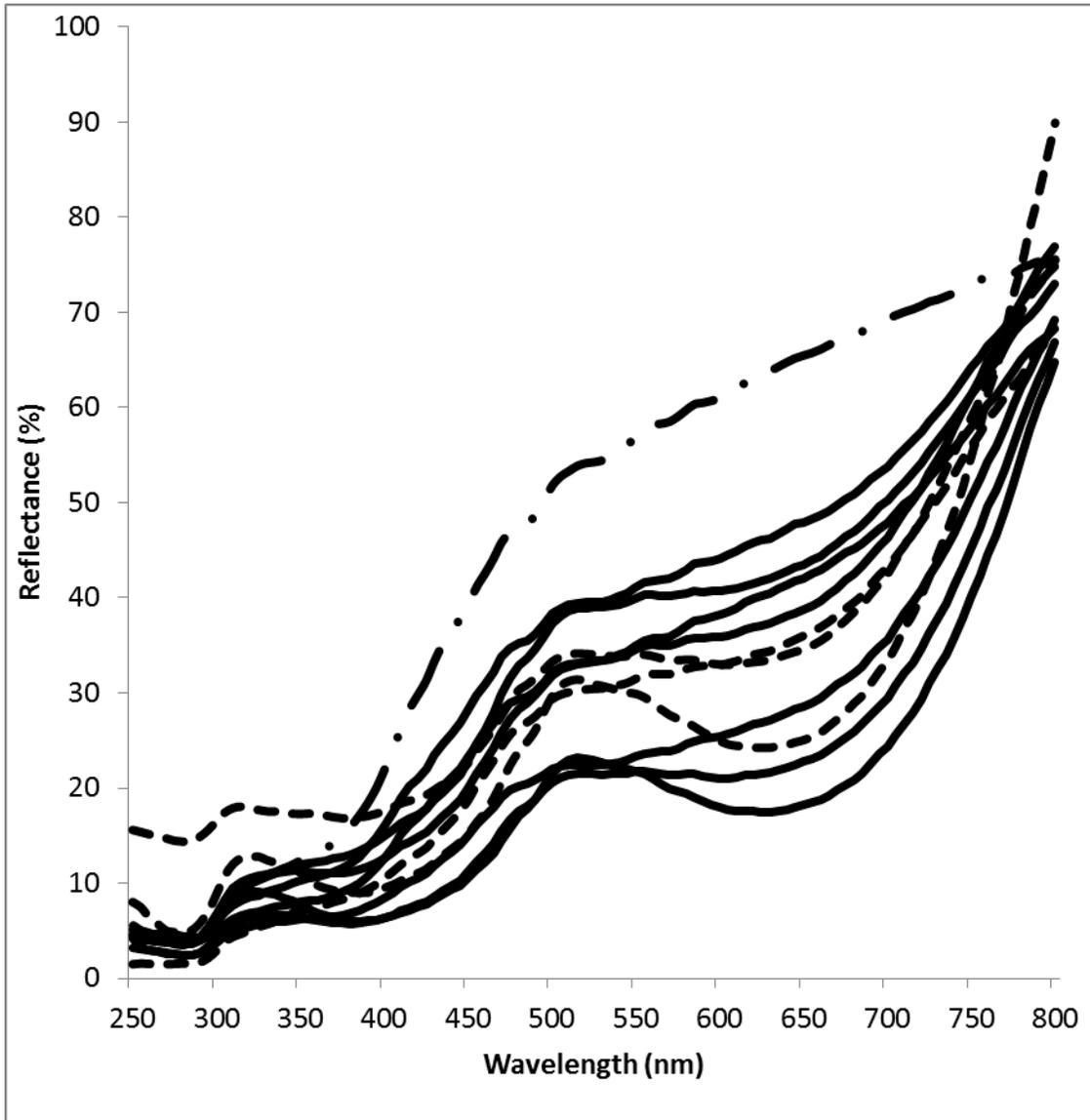


Figure 5. Cassowary egg spectra, variation among species and individuals. All three extant species are depicted: the southern or double-wattled cassowary *Casuarium casuarium* (seven solid lines), the dwarf or Bennett's cassowary *C. bennetti* (three dashed lines), and the northern or single-wattled cassowary *C. uniappendiculatus* (dashed and dotted line). Each line represents one egg of a distinct individual with the exception of two *C. casuarium* lines, each of which represent the average of a four-egg clutch.

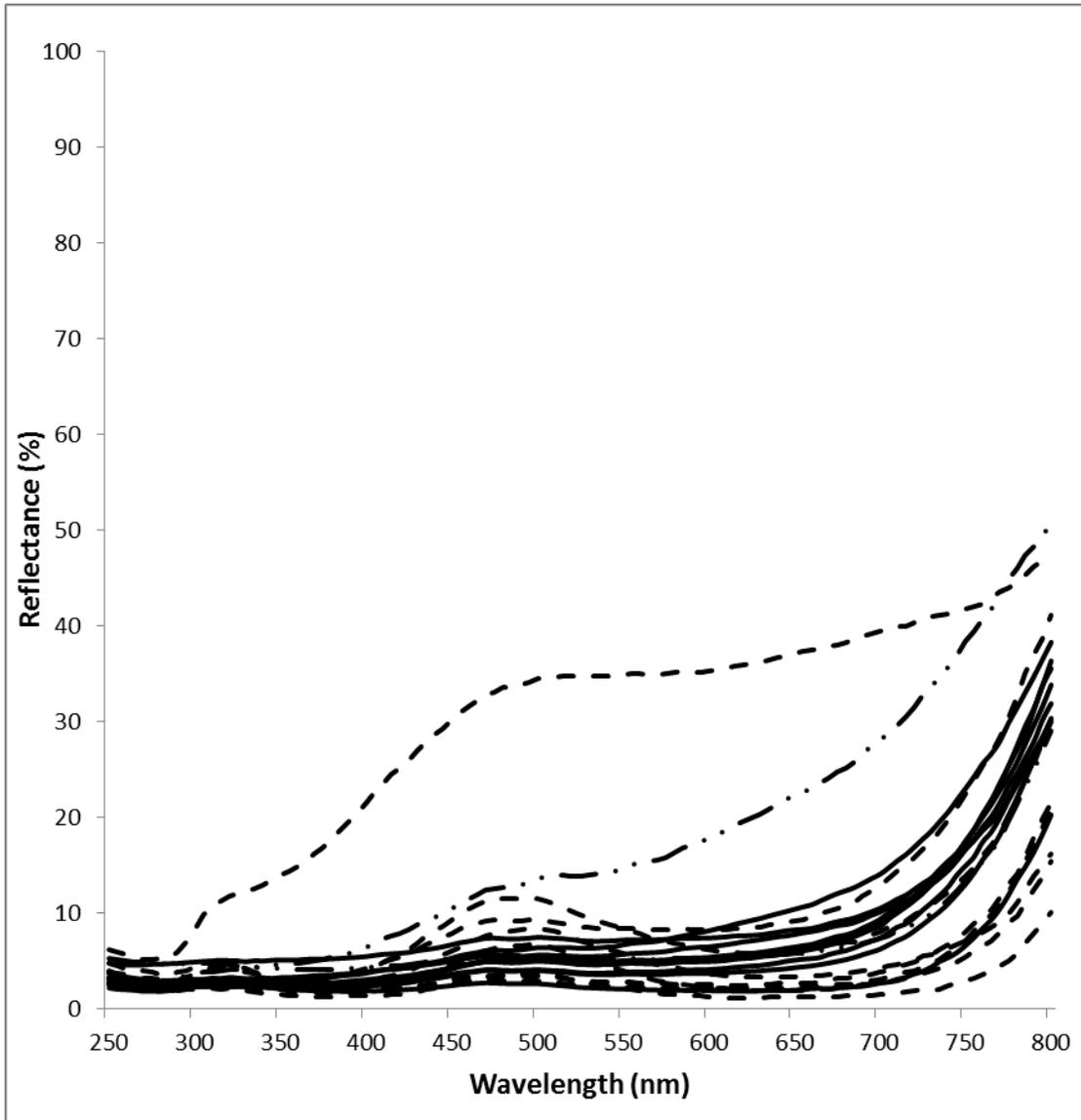


Figure 6. Emu egg spectra, variation among taxa. The emu *Dromaius novaehollandiae novaehollandiae* is depicted (nine solid lines-wild, eight dashed lines-captive), along with an occasionally purported second species, the spotted emu *Dromaius irroratus* (two long dash dot dot lines). Each line represents an individual egg in a single clutch with the exception of wild *Dromaius novaehollandiae novaehollandiae* which represents an average of an eight-egg clutch and *Dromaius irroratus* representing an average of a nine-egg clutch.

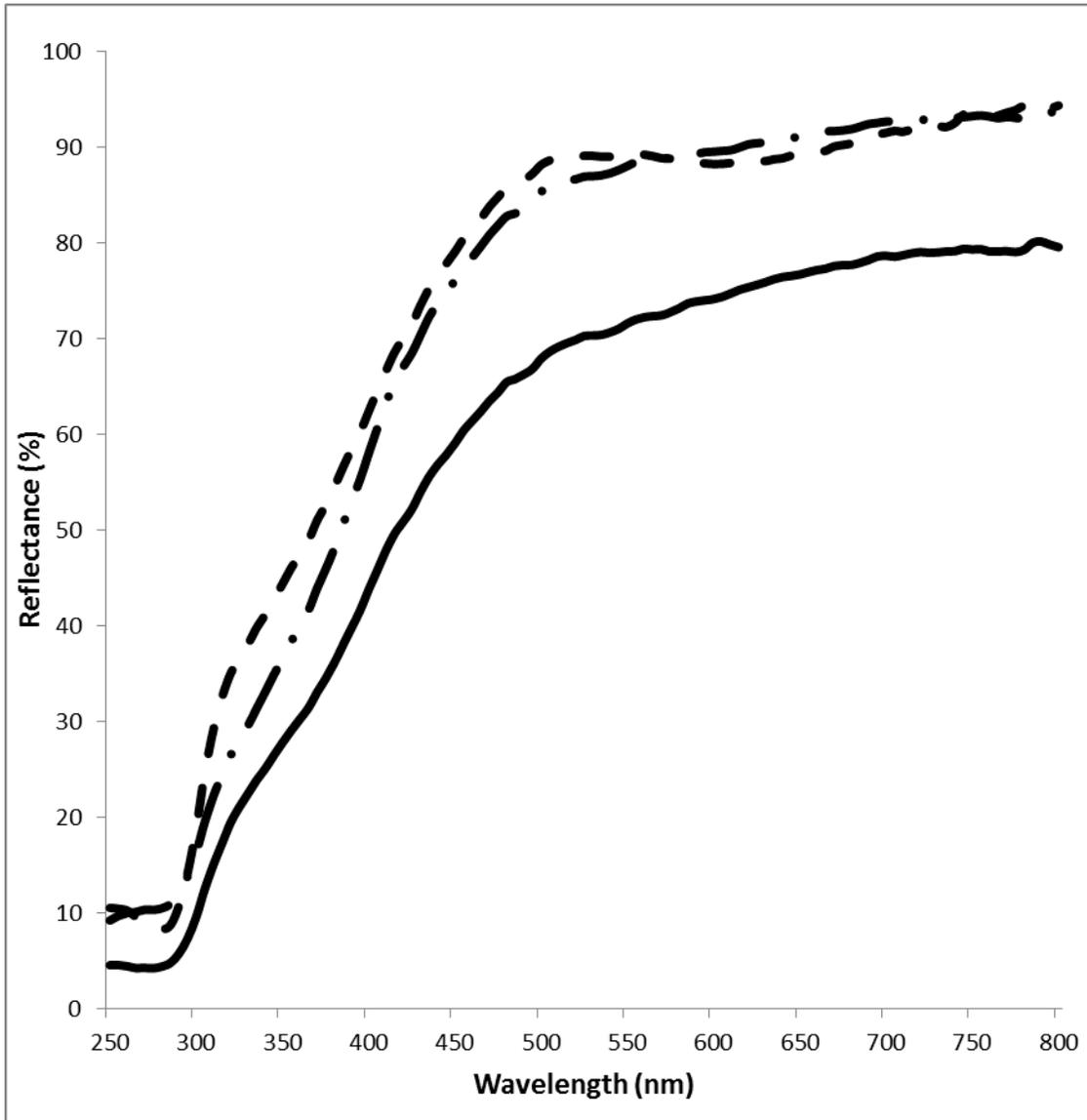


Figure 7. Kiwi egg spectra, variation between species and individuals. Two species are depicted: the North Island brown kiwi *Apteryx mantelli* (solid line-wild, dashed line-captive) and the southern brown kiwi or southern tokoeka *Apteryx australis* (dashed and dotted line).

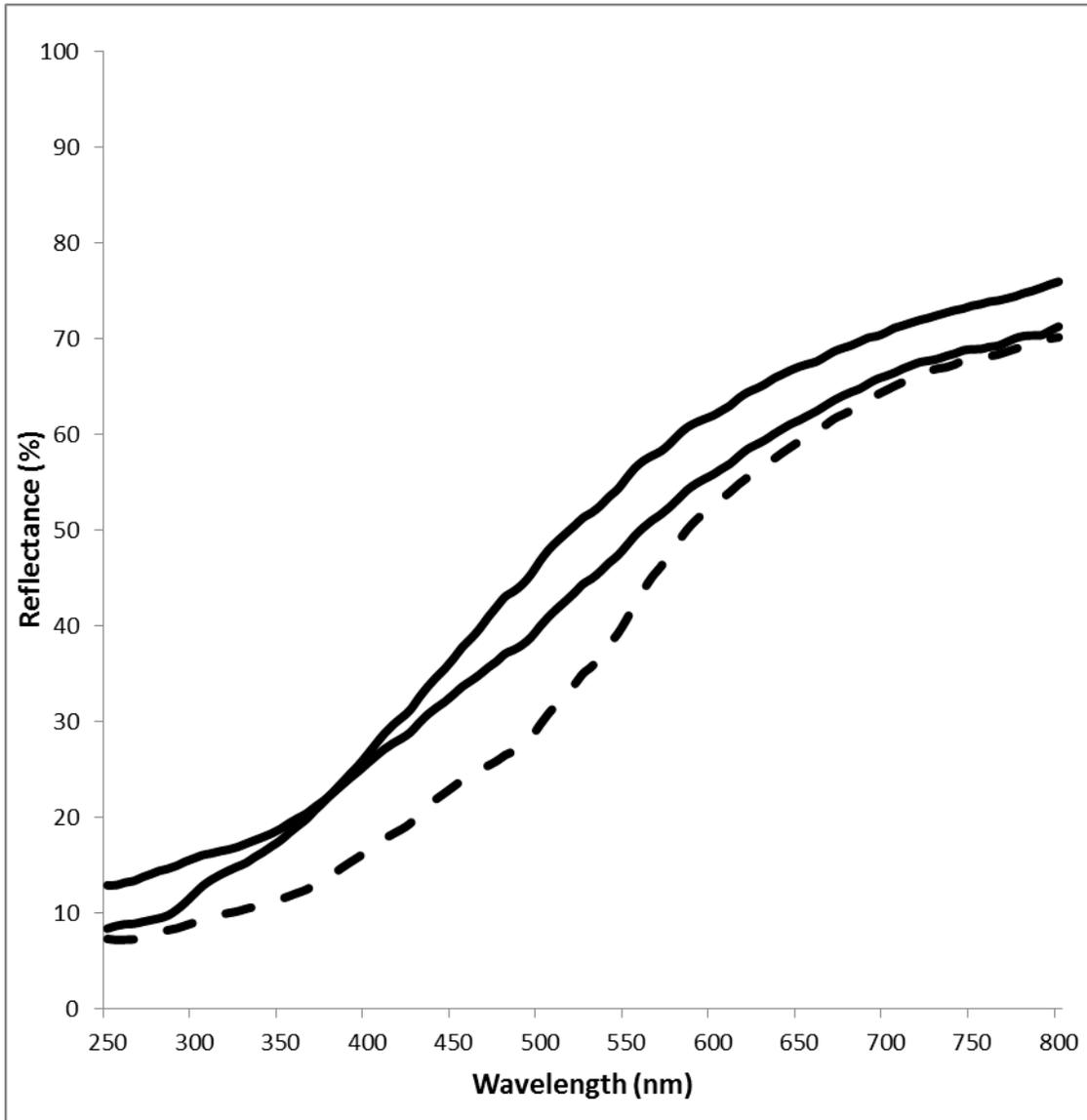


Figure 8. Elephant bird (*Aepyornis maximus*) egg spectra, variation among individuals. Each line depicts an average of four reads of a single egg. The dashed line represents an egg that was never opened (still contains the remains of an embryo).

PLATES

1. Egg of the common ostrich, *Struthio camelus*
2. Egg of the greater (common) rhea, *Rhea americana albescens*
3. Egg of the one-wattled cassowary, *Casuarius uniappendiculatus*
4. Egg of the emu, *Dromaius novahollandiae*
5. Egg of the common kiwi, *Apteryx australis*
6. Egg of the elephant bird, *Aepyornis maximus*

All photos of eggs from the Western Foundation of Vertebrate Zoology, Camarillo, California, U.S.A. Photography by René Corado.

1

WESTERN FOUNDATION
OF VERTEBRATE ZOOLOGY
A.O.U. No. _____ Set No. 11,251-22
Ostrich
Struthio camelus massaicus
Loc. nr. Talek River, S. Masai, Kenya
Date 22 Sept 1953 Coll. E.N. Harrison



2

WESTERN FOUNDATION OF VERTEBRATE ZOOLOG
Set Mark: Data Cat. No.: 188804-1
Greater (Common) Rhea
Rhea americana albescens
Loc.: Barrio Blanco, Rio Negro, Argentina
Date: 14 Nov 1904 Coll.: Not recorded



3

ED N. HARRISON
OOLOGICAL COLLECTION
A.O.U. No. Set No. 22515
ONE-WATTLED CASSOWARY
CASUARIUS UNIAPPENDICUATUS
Loc GERMAN NEW GUINEA ID OF SALAWATI
Date Coll. by H. HOCKE



4

WESTERN FOUNDATION
OF VERTEBRATE ZOOLOGY
A.O.U. No. _____ Set No. 165,093-7
Emu.
Dromaius novahollandiae
Loc. Captive Camarillo CA.
Date 9 Feb 1997 Coll. Lilian Eichner



5

WESTERN FOUNDATION
OF VERTEBRATE ZOOLOGY
A.O.U. No. _____ Set No. 148,239-1
Common Kiwi
Apteryx australis
Loc. New Zealand
Date 1890 Coll. of Thompson



6

WESTERN FOUNDATION
OF VERTEBRATE ZOOLOGY
ADU No. _____ Set No. 147.660-1
Elephant Bird
Aepyornis maximus
Loc. Fort Dauphin, Madagascar
Date 1910 Coll. of R.D. Etchecopar

