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Reflectance and artificial nest experiments of reptile and bird eggs imply an adaptation of bird eggs against ultraviolet

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Abstract Interspecific comparative studies in trait responses to nest predation will help uncover potential costs associated with changes in nest predation risk that may constrain the degree of the response. Reptiles build large mounds of sand, soil or compost in which they bury and leave their eggs after laying. As their eggs are not exposed, reptiles consistently lay immaculate white eggs; while some birds that also lay immaculate white eggs place their nests in exposed sites under sunlight. Comparing the spectrum of reptile and bird eggs, especially in the ultraviolet (UV) region, may help us understand the evolution of coloration in bird eggs. We measured white immaculate eggs from four species of turtle and three species of birds by spectrometer, and compared their hue, chroma, UV brightness, and total brightness. Our results indicated that the UV reflectance of bird eggs consistently exhibited peaks and troughs in waveform that obviously differed from that of turtle eggs, which rising gradually from 300 to 400 nm. This finding implies that bird eggs have been released from enclosed nests, like those of reptiles, and have thus evolved an adaptation in UV reflectance as a signal. Furthermore, artificial nest experiments indicated that predation rates of bird eggs were significantly higher than those of turtle eggs, implying that such a signal might exist. Our study provides baseline data and perspective for further research on the evolution of egg coloration between reptiles and birds.

Keywords Bird egg · Nest predation · Turtle egg · Ultraviolet spectrum · UV reflectance

Introduction

As one of the most important selective pressures, predation plays a key role in shaping evolutionary relationships in nature (Caro 2005). Therefore, differences in antipredator responses between ecological and evolutionary contexts deserve more attention. Interspecific comparative studies in trait responses to nest predation will complement our general understanding of factors influencing phenotypic plasticity of prey (review in Ibáñez-Álamo et al. 2015). For example, a comparative approach can help uncover potential costs associated with changes in nest predation risk that may constrain the degree of the response (Ibáñez-Álamo et al. 2015). However, studying nest predation in other bird groups beyond passerines or other geographical areas different from the most commonly studied forested temperate regions remains a challenge (Ibáñez-Álamo et al. 2015).

Recently a phylogenomic study supported the idea that the turtle is a sister group of the birds (Chiari et al. 2012), implying a feasible comparison for morphological traits that help explain evolutionary phenotypic plasticity. Reptiles build large mounds of sand, soil or compost in which they bury and leave their eggs after laying (Deeming and Ferguson 1991). As their eggs are not exposed, reptiles consistently lay immaculate white eggs. In contrast, birds build open nests exposed to sunlight and their eggs have remarkable variation in color. Bird eggs vary considerably among species in the color of their shells and the patterns that adorn them. They may be white or red, violet or chocolate brown, glossy turquoise or emerald green; and they may be immaculate, or covered in dense markings (Kilner 2006). Because reptile eggs are pure white, and hole-nesting birds are more likely to lay immaculate white eggs, Wallace (1889) proposed a hypothesis that the ancestral egg was white and that all other forms of egg color and patterning were adaptations to the specific microenvironment of each nest, functioning to conceal eggs from predators. However, not all birds whose nests are placed in exposed sites lay eggs with non-white color

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or patterning. Birds that possess UV-sensitive photoreceptors show widespread sensitivity to ultraviolet (UV) light, perceiving it and using it in food selection (Church et al. 1998), mate choice (Siitari and Huhta 2002), parent-offspring interactions (Jourdie et al. 2004) and egg discrimination (Yang et al. 2013). Therefore, detailed information about the difference between reptiles and birds is important, and comparing the spectrum between reptile and bird eggs—especially in the UV spectrum—may help us understand the evolution of coloration in bird eggs.

Compared with reptiles, birds are UV-sensitive and bird eggs are functionally informative (Bennett and Cuthill 1994). In addition, Bertram and Burger (1981) argued that egg pigmentation would provide benefit in the form of crypsis, but it can also carry an associated risk that the egg would overheat when in direct sunlight. Previous studies have indicated that predators may use UV light as a cue for prey searching (Bennett and Cuthill 1994; Hokavaara et al. 2002), and a recent field study by Yang et al. (2015) showed that blocking UV reflectance of bird eggs significantly decreased their predation rates by aerial predators. Therefore, birds that lay their eggs in exposed nests are confront a trade-off between the risk of predation from UV-sensitive predators and overheating from UV light (due to overly dense pigmentation).

Here we compared the reflectance spectrum between reptile eggs (four turtle species belong to Testudines as representatives of reptiles) and bird eggs (three avian species belong to Galliformes, Columbiformes and Passeriformes as representatives of birds; see Table 1 for details). These three species of birds all build open nests with eggs exposed to sunlight. All turtle and bird eggs from selected species in the study are immaculate white according to human eyes. We predicted that UV reflectance of bird eggs should differ from that of turtle eggs in some aspects. We also conducted an artificial nest experiment to compare the predation rates between bird eggs and turtle eggs.

Materials and methods

Egg color quantification

The color of the turtle and bird eggs was measured using a spectrophotometer (Avantes-2048, Avantes, Apeldoorn,

The Netherlands), with a halogen light source (Avalight-Hal-S) and a coaxial reflectance probe (FCR-7UV200-2-ME). Egg color reflectance was measured under total darkness of a light cover to avoid the influence of ambient light and standard light conditions. Light reflectance intensity was expressed relative to a 99 % white reflection standard (WS-1). Each measurement covered approximately 1 mm² and was taken at a 45° angle to the egg surface with the reflectance probe fixed by a probe holder (RPH-1). A total of six stratified random samples were taken per egg and its reflectance was summarized as the mean of six measurements (two at the blunt, two at the middle, and two at the sharp parts of the egg, see Yang et al. 2010). Because turtle and bird eggs used in this study are uniformly white (i.e. each egg was covered by uniform pigmentation), we averaged the six spectra for each egg. Previous studies have indicated a high repeatability of spectrophotometric measurements, even for spotted eggs (Avilés et al. 2006, 2010). For each sample measurement, the average and integration time were set to 20 times and 100 ms, respectively. We used Goldsmith's (1990) tetrahedral color space to analyze egg coloration. The spectra were loaded into Ava-Soft 7.0 software and interpolated with a step of 1 nm in the range 300–700 nm (Yang et al. 2010, 2012). Eggs were collected from many different individuals of turtles and birds to avoid pseudoreplication.

Artificial nest experiment

Local plant branches and leaves were used to build artificial nests, in which two pigeon eggs (domestic pigeon *Columba livia domestica*) or two turtle eggs (Chinese stripe-necked turtle *Mauremys sinensis*) were included to represent artificial bird nests or turtle nests, respectively (Fig. 1). Bird nests and turtle nests were set up on shrubs in a tropical forest of Diaoluoshan National Nature Reserve in Hainan Island of China, with similar height (mean rank: 55.1 vs. 45.8 cm, $Z = -1.605$, $P = 0.109$, Mann–Whitney U test) and cover (mean rank: 48.5 vs. 52.4 %, $Z = -0.693$, $P = 0.488$, Mann–Whitney U test), and with an alternative position and a constant distance of 5 m from each other. The cover of artificial nests was evaluated using human vision by the same observer (Nan Niu). Nests

Table 1 Species of reptile and bird eggs used for spectral analysis. N was the sample size for each species

Species		Taxonomy			N
English names	Latin names	Classes	Orders	Families	
Chinese stripe-necked turtle	<i>Mauremys sinensis</i>	Reptilia	Testudines	Geoemydidae	30
Chinese pond turtle	<i>Mauremys reevesii</i>	Reptilia	Testudines	Geoemydidae	30
Chinese softshell turtle	<i>Pelodiscus sinensis</i>	Reptilia	Testudines	Trionychidae	30
Red-eared slider	<i>Trachemys scripta elegans</i>	Reptilia	Testudines	Emyidae	30
Chicken	<i>Gallus gallus domesticus</i>	Aves	Galliformes	Phasianidae	30
Domestic pigeon	<i>Columba livia domestica</i>	Aves	Columbiformes	Columbidae	16
Ashy-throated parrotbill	<i>Paradoxornis alphonianus</i>	Aves	Passeriformes	Sylviidae	9

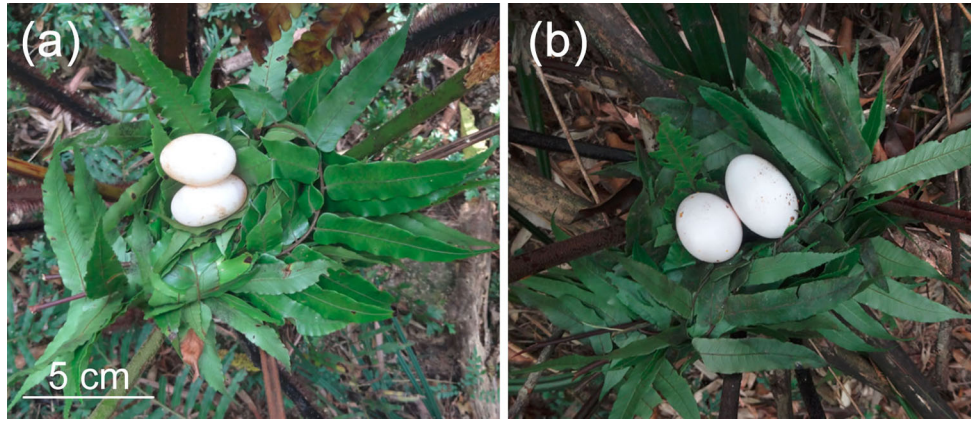


Fig. 1 Experimental nests on shrubs used in this study, in which two turtle eggs (a Chinese stripe-necked turtle *M. sinensis*) or two pigeon eggs (b domestic pigeon *C. l. domestica*) were included to represent artificial turtle nests or bird nests, respectively

were checked on the first, third, sixth, ninth, and twelfth day after placement to investigate predation status. The artificial nests in this study were bird eggs or turtle eggs used for the experiment rather than simulating real bird nests or turtle nests, because in nature there is no combination of turtle eggs with such nest structures.

Statistical analysis

We used the following equations to calculate the hue, chroma, total brightness and UV brightness of eggs (Endler 1990; Yang and Liang 2013).

$$\text{chroma} = \sqrt{(R - G)^2 + (Y - B)^2}$$

and

$$\text{hue} = \arcsin\left(\frac{Y - B}{\text{chroma}}\right),$$

where B, G, Y, and R refer to the reflectance sum of blue (400–475 nm), green (475–550 nm), yellow (550–625 nm), and red (625–700 nm) regions of visible light. Furthermore, the total brightness was calculated by the total sum of reflectance from 300 to 700 nm while the UV brightness was obtained by the sum of reflectance from 300 to 400 nm. Statistical analyses were performed in SPSS 13.0 for Windows (SPSS Inc., Chicago, IL, USA). MANOVA was used to compare the color parameters among eggs of different species while LSD (least squares difference) was used to perform a pairwise comparison. Nest predation rates between bird eggs and turtle eggs were compared by Chi square test. All statistical tests were two-tailed, and data are presented as mean \pm SE.

Results

In total, we obtained reflectance spectra for 120 turtle eggs (30 for each species) and 55 bird eggs (30 for pi-

geon, 16 for parrotbill and 9 for chicken). Egg reflectance from 300 to 700 nm is presented in Fig. 2 for all species. Although the egg spectra curves were different among different species, the UV spectrum (300–400 nm) of bird eggs consistently exhibited peaks and troughs of waveform that obviously differed from that of turtle eggs (rising gradually in 300–400 nm without peaks and troughs). All color parameters of eggs were significantly different among species (chroma: $F = 11.3$; hue: $F = 70.8$; UV brightness: $F = 25.2$; total brightness: $F = 30.1$; $df = 6$ and $P < 0.01$ for all, MANOVA). For more details about pairwise comparison see Fig. 3.

For the artificial nest experiment, a total of 100 nests were placed and the predation rates differed over days between bird eggs (50 nests) and turtle eggs (50 nests; Fig. 3). All the turtle eggs in artificial nests were intact while the bird eggs were predated and the predation rates increased with exposed days. On the twelfth day the predation rate of bird eggs was significantly higher than that of turtle eggs (Chi square = 4.167, $df = 1$, $P = 0.041$; Fig. 4).

Discussion

Our results indicated that the UV reflectance of bird eggs consistently exhibited peaks and troughs in waveform that obviously differed from that of turtle eggs, which rose gradually from 300 to 400 nm. Further, the artificial nest experiment showed that the predation rate of bird eggs was significantly higher than that of turtle eggs.

There are several hypotheses that attempt to explain the evolution of egg color and patterning in birds: (1) the crypsis hypothesis, which suggests that the ancestral egg is white and that all other forms of egg color and patterning are adaptations to the specific microenvironment of each nest, functioning to conceal eggs from predators (Wallace 1889); (2) the aposematic hypothesis, which

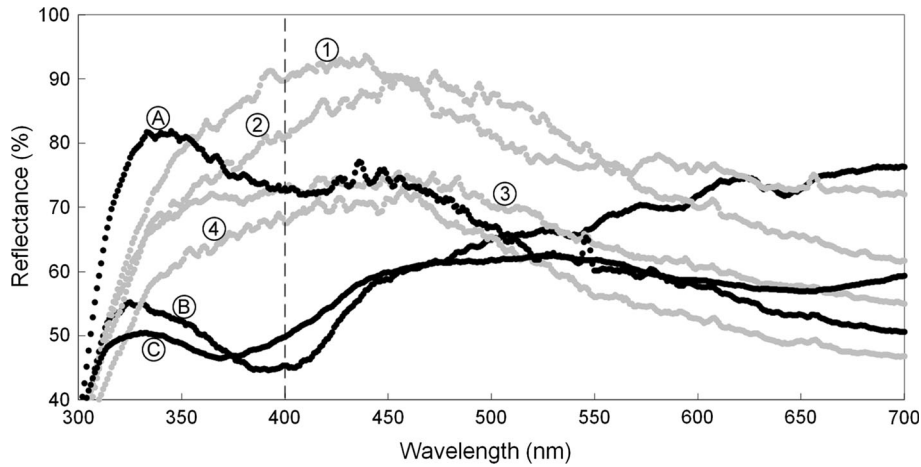


Fig. 2 Averaged egg reflectance of turtle and bird species in this study. *Black and grey curves* refer to bird and turtle eggs, respectively. *Dashed lines* separate the ultraviolet (300–400 nm) and visible (400–700 nm) zones of light. *Letters and numbers* indicate the species names of birds and turtles, respectively.

A domestic pigeon (*C. l. domestica*), *B* chicken (*Gallus gallus domesticus*), *C* ashy-throated parrotbill (*P. alphonsianus*), *1* red-eared slider (*Trachemys scripta elegans*), *2* Chinese stripe-necked turtle (*M. sinensis*), *3* Chinese softshell turtle (*Pelodiscus sinensis*), *4* Chinese pond turtle (*Mauremys reevesii*)

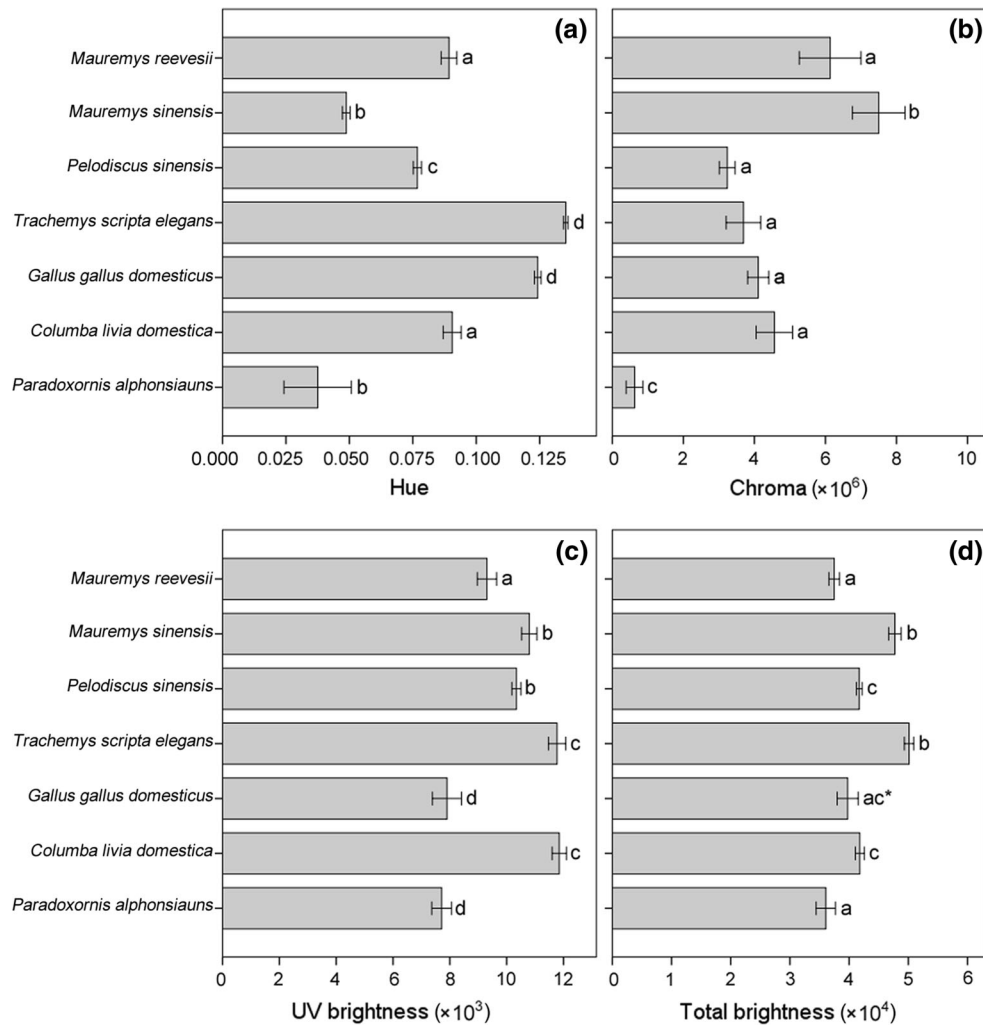


Fig. 3 Comparison of hue, chroma, UV brightness and total brightness in different species by least squared difference (LSD). *Different or identical letters* indicate that statistical significance was

reached (i.e. $P < 0.05$) or not, respectively. *Significant level of total brightness of chicken eggs was the same as either “a” or “c” (i.e. between “a” and “c”)

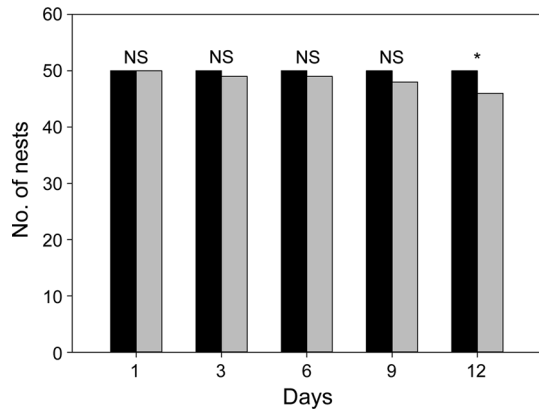


Fig. 4 Intact numbers of nests and days from the start of artificial nest experiment. *Black* and *grey* bars refer to the bird nests and turtle nests, respectively, in which the eggs were intact when checked (i.e., bar length decreases as predation rate increases). NS and * refer to $P > 0.05$ and $P < 0.05$, respectively

suggests that colorful eggs are aposematic and advertise their unpalatability to any potential predator (Swynnerton 1916); (3) the female quality hypothesis, which suggests that blue egg coloring may be selected by male birds, which are keen to assess the quality of parental investment offered by their partner, and that they might adjust their contribution of offspring care accordingly (Moreno and Osorno 2003); (4) the thermoregulation hypothesis, which suggests that different egg coloration may be an adaptation to thermoregulation under different environmental temperatures (Montevecchi 1976); (5) the brood parasitism hypothesis, in which egg color evolution is driven by the coevolutionary interaction between brood parasites and hosts, under the selection of egg mimicry from parasites and egg rejection from their hosts (Davies 2000).

The crypsis hypothesis reasonably explains egg color in many species of birds. However, there are about 20 % of birds (including pigeons) whose nests are placed in exposed sites even when eggs are immaculate white (Kilner 2006). Under the hypothesis of aposematism, some investigators have tried to prove that colorful eggs are more unpalatable (Swynnerton 1916; Cott 1948, 1952). However, essentially no evidence was found to support this hypothesis, and no toxic eggs have been identified in birds so far. For the female quality hypothesis, previous studies have suggested that blue eggs evolved specifically to signal female quality (Moreno and Osorno 2003; Soler et al. 2005; but see Kilner 2006). The thermoregulation hypothesis has been tested by two empirical studies (Montevecchi 1976; Bertram and Burger 1981). Although these studies found support for this hypothesis, all of them focused on ground-nesting birds. In the brood parasitism hypothesis, white egg color may have evolved in hosts to counteract brood parasitism. Yang et al. (2010) presented strong experimental evidence that parrotbills (*Paradoxornis alphonisianus*) in mainland China lay polymorphic eggs in

white, pale blue, and blue colors to escape brood parasitism, while the common cuckoo (*Cuculus canorus*) evolved corresponding egg colors as a consequence of frequency-dependence. In contrast, the common cuckoo is absent in Taiwan and the parrotbill population there lays monomorphic blue eggs, which implies that white egg color is an adaptation to brood parasitism. However, pigeons are not suitable hosts for brood parasites because they feed their offspring by using pigeon “milk”, which cannot be used to rear cuckoo nestlings successfully (Davies 2000). Therefore, the evolution of pigeon egg color has no history of interaction with avian brood parasites.

Recent studies using artificial nests with chicken eggs (Wang et al. 2014) and turtle eggs of *Cuora galbinifrons* (Li et al. 2014) showed that predation on the ground nest eggs of birds (72.7 % by mammals) was higher than that of turtle underground nests (51.3 % by mammals). Previous studies also showed that UV reflectance of bird eggs possess some information functioning such as for egg discrimination (Yang et al. 2013). Although our finding could not provide direct evidence to support any of the hypotheses mentioned above, it implies that the characteristics of UV reflectance on bird eggs may play some role in information transfer compared with reptile eggs. Additionally, it implies that UV reflectance in bird eggs has evolved in the presence of UV as an adaptation against over-heating, which in turn can be detected by aerial predators searching for nests. In other words, bird eggs have been released from enclosed nests, like those of reptiles, and have thus evolved the adaptation of UV reflectance as an informative agent. Previous studies indicated that predators may use UV light as a cue for prey searching (Bennett and Cuthill 1994; Hokavaara et al. 2002), and Yang et al. (2015) showed that blocking UV reflectance of bird eggs significantly decreased their predation rates by aerial predators. In the present study, bird eggs exhibited peaks and troughs of waveform in their UV reflectance, although the UV brightness of bird eggs was equal to or significantly lower than that of turtle eggs (Fig. 2). Furthermore, the artificial nest experiment revealed that the predation rate of bird eggs was significantly higher than that of turtle eggs, implying that UV reflectance of bird eggs might contain some kind of information that may be detected by aerial predators. In summary, this study provides baseline data and perspective for further research on the evolution of egg coloration between reptiles and birds.

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Compliance with ethical standards

Conflict of interest We declare that all authors have no conflict of interest.

Ethical standard The experiments comply with the current laws of China in which they were performed. Experimental procedures were in agreement with the Animal Research Ethics Committee of Hainan Provincial Education Centre for Ecology and Environment, Hainan Normal University. Fieldwork was carried out under the permission from Diaoluoshan National Nature Reserve, China.

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