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Do weather conditions affect the colouration of great reed warbler *Acrocephalus arundinaceus* eggs?

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Abstract. The goal of this paper was to test the hypothesis that weather conditions, such as temperature and rainfall, affect egg colouration in the great reed warbler *Acrocephalus arundinaceus*. We failed to find strong support for this hypothesis; nevertheless, our results indicate that temperature has an effect on some egg colour characteristics. Eggshell brightness (PC1) increased with increasing temperature at laying and eggs were darker in the colder year of the two-year study. On the other hand, UV-blue colouration (PC2) scores were higher in the warmer year. The amount of rainfall, however, had no effect on eggshell colouration. As there is an indication from other studies that weather may have an effect on egg appearance through the food availability, we encourage further testing the environmental hypothesis in other bird species. If this holds for more bird species, this would have important implications for the hypotheses about the adaptive function of bright eggshell colouration.

Key words: egg appearance, passerines, precipitation, temperature, UV reflectance

Introduction

The function and origin of avian eggshell colouration have puzzled evolutionary biologists for more than a hundred years. Since the pioneering work of Wallace (1890) and Newton (1896), a growing body of studies has stimulated researchers to review this topic carefully during the last decade (Underwood & Sealy 2002, Kilner 2006, Cherry & Gosler 2010, Maurer et al. 2011). At the proximate level, eggshell pigments are responsible for colouration (Solomon 1997). At the ultimate level, egg appearance serves a variety of tasks, including protection, signalling and/ or communication. For example, Lahti (2008) showed that some pigments may protect embryos by filtering harmful solar radiation. Moreno & Osorno (2003) proposed that egg colouration is a sexually selected signal of female quality to males, but empirical studies have found mixed support for this hypothesis (Cherry & Gosler 2010, Honza et al. 2011, Riehl 2011). Egg appearance is also crucial in the detection of both intraspecific (Lahti & Lahti 2002, Ležalová-Piálková

& Honza 2008, López-de-Hierro & Moreno-Rueda 2010) and interspecific brood parasitism (Moskát & Honza 2002) and, cryptic egg colouration may minimise the probability of depredation (Underwood & Sealy 2002, Kilner 2006).

Although most authors have emphasised that the observed variability in egg colours and patterning is mostly evolutionary induced (Goodman & Shealy 1977, Collias 1993, Mikšík et al. 1996, Lahti 2008), there is some evidence that various environmental factors might influence the biosynthesis of eggshell pigments and, consequently, eggshell appearance (Gosler et al. 2000, Jagannath et al. 2008). It has also been shown that ground colour may reflect female condition (Siefferman et al. 2006) and egg colour may change as a function of stress (Arendt 2004).

For example, Gosler et al. (2005) referred that eggshell maculation varies with the availability of calcium, an important dietary component. Weather conditions during the breeding season may also influence food quality and availability. A link between these factors

and various developmental stages of different insect taxa can affect egg colouration as suggested by Hargitai et al. (2008) for collared flycatchers *Ficedula albicollis*. The assumption of diet-mediated influence on egg appearance was supported by an experimental study by Moreno et al. (2006), who found that closely related pied flycatcher *Ficedula hypoleuca* females supplemented with food prior to and during laying laid more intensely blue-green eggs than non-supplemented females.

Recently, Avilés et al. (2007) provided support for the hypothesis that environmental conditions may affect phenotypic egg matching between common cuckoo *Cuculus canorus* and host eggs. They found that Eurasian reed warbler *Acrocephalus scirpaceus* eggs were more brilliant in springs with higher rainfall and tended to be bluer and greener in springs with lower relative temperatures. However, their study was based only on museum egg collections (for drawbacks of this approach see Cassey et al. 2010). If their findings have general validity, then one should expect that weather factors, like temperature and/or rainfall, should indirectly affect egg colouration through food availability. Egg colouration should therefore show wide variation among breeding attempts, which would have important evolutionary consequences for the various signalling functions of eggshell colouration.

Natural selection can only operate on the component of eggshell colouration that is genetically determined. Therefore, studies that document environmental influences are valuable because they help us evaluate hypotheses for the adaptive value of bright eggshell colours. For example, if male birds are using the colour of the eggshell to judge the quality of their mates (as proposed by the sexually selected hypothesis; Moreno & Osorno 2003), then environmentally induced changes in eggshell colouration would operate against this. The major goal of this study was therefore to test the hypothesis that environmental conditions affect egg colouration in the great reed warbler *Acrocephalus arundinaceus* within the breeding season. As this species is closely related to the Eurasian reed warbler and occupies a similar trophic niche, we expected great reed warbler eggs to be bluer and greener in wetter and colder periods, respectively, and their brightness to be positively influenced by the amount of precipitation during egg laying, in accordance with Avilés et al. (2007).

Methods

Study species

The great reed warbler is a migratory passerine breeding in wetlands of the Western Palaearctic. It typically lays one clutch of five eggs per year. Its diet is composed mainly of insects, mostly dragonflies, dipterans, homopterans, and beetles, with molluscs and arachnids representing additional prey (Cramp 1992). Great reed warblers lay pale green, blue or blue-green (in background colour) eggs speckled and blotched olive green, pale blue-grey and dark brown (Cramp 1992). Indeed, spectrophotometry measurements showed that the eggs exhibited proportionally higher reflectance in the blue-green portion of the spectrum (with a peak between 490 and 580 nm; Honza et al. 2011), which corresponds to their general blue-green appearance to the human eye.

Fieldwork

The study was conducted on a colour-ringed great reed warbler population in two adjacent fishpond areas between Hodonín (48°51′ N, 17°07′ E) and Mutěnice (48°54″ N, 17°02′ E), Czech Republic, from late April to mid July 2008 and 2009. We searched for nests in littoral vegetation surrounding the fishponds. Most of them were found during the building stage or at the beginning of egg laying and were checked daily to record the laying date and clutch size.

After clutch completion, spectral reflectance of eggs was measured with a spectrophotometer (range: 300-700 nm; USB2000, Ocean Optics), a deuterium-halogen light source, and a fibre-optic probe. The measurements were taken from a 1 mm^2 region of the eggshell surface at an angle of 45° under standard light conditions and were relative to a white reference (WS-2 Spectralon, Ocean Optics) and to the dark. Reference and dark calibration were made prior to the measurement of each clutch. Data from the spectrophotometer were loaded into OOIBase 32 (Ocean Optics) software. To prevent nest desertion during the measurements, we temporarily exchanged the host clutch with four to five conspecific eggs collected from abandoned nests. We took nine measurements across the eggshell which represented both the background colouration and the basic colour features of the complex spotting pattern of great reed warbler eggs (as in Polačiková et al. 2009).

Data analysis

We performed a principal component analysis (PCA) on mean reflectance spectra per clutch to reduce the large number of correlated variables (i.e. reflectances at each 1.75 nm interval) into a few orthogonal variables that summarised most of the variation (Cherry et al. 2007). We selected only the principal components (PC) that complied with Kaiser's criterion (eigenvalues > 1).
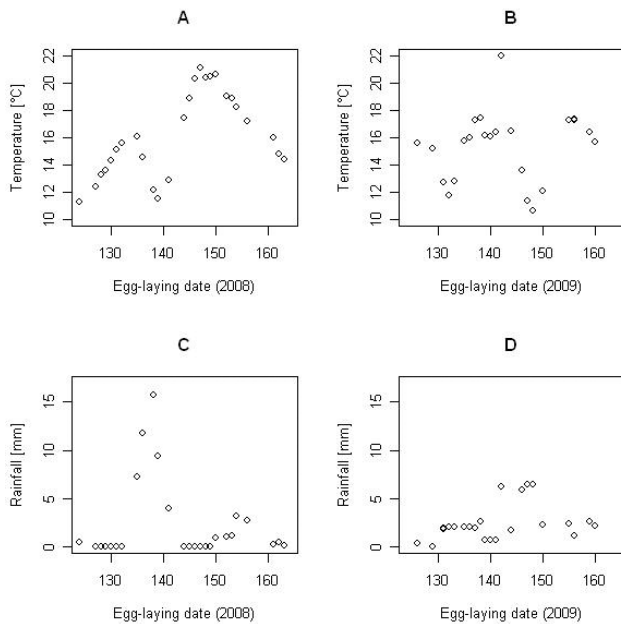
Fig. 1. Daily temperature and rainfall (averaged for the egg-laying period of each clutch) in the course of the two study years. Egg-laying date is the Julian date of the first egg laid in the clutch.

Data on mean daily amount of precipitation were obtained from the nearby meteorological station in Dubňany (located 4 km from the study site) and mean daily temperatures from the meteorological station in Strážnice (17 km from the study site). For each clutch, we calculated mean values for these two parameters from the first to the fifth day of egg laying to cover the time window when the female synthesises eggshell pigments (Warren & Conrad 1942, Woodard & Mater 1964).

To explore the effect of precipitation and temperature on egg colouration, we fitted two sets of general linear
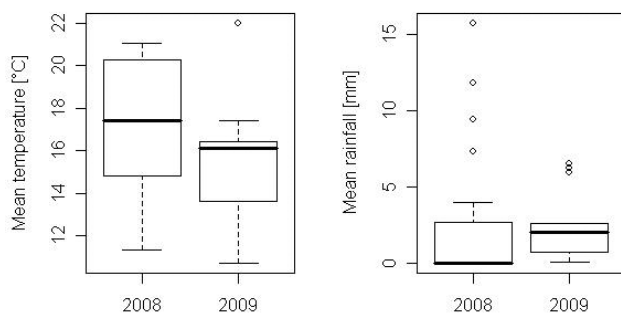

Fig. 2. Mean daily temperature and rainfall (averaged for the egg-laying period of each clutch) in the two study years. The central bar of the box indicates the median, the box delimits 25th and 75th percentiles, whiskers show the most extreme data points within 1.5 times the interquartile range and circles denote outliers. For tests see the Results.

models (GLMs) with the principal components as a response variable and mean values of precipitation, mean temperature, year and laying date as predictors. To avoid pseudoreplications, only one clutch per each female (the first measured) was used in the analyses. Clutches where we did not know the identity of the females were not used. A total of 116 measured clutches were used for analysis (62 in 2008 and 54 in 2009).

Model selection was based on Akaike's information criterion (AIC), corrected for low sample size (AIC$_c$) following the recommendations of Burnham & Anderson (2002). Models were ranked from the best to the worst using Δ_i ($\Delta_i = AIC_{c(i)} - AIC_{c(min)}$) and the Akaike weights (w_i) were calculated to give the relative support for a given model compared with the others. For the top candidate models that provided substantial support ($\Delta_i \leq 2$), we applied model averaging to identify the relative importance of each model term in predicting the response variable and to estimate robust model parameters.

PCA was performed in STATISTICA 6.0; all other statistical analyses were conducted in R 2.13.1 (R Development Core Team 2011). Model selection and averaging procedures were done in the R package MuMIn (Bartoń 2011).

Results

Timing of breeding and weather conditions

There were no differences in the initiation date of measured clutches between the two years of the study (the mean clutch initiation date in both years: 23 May ± 10 and 9 days, for 2008 and 2009, respectively; t-test: $t = 0.18$, $P = 0.86$, $n_1 = 62$, $n_2 = 54$, respectively). Both temperature and rainfall at laying varied in the course of the season in both years (Fig. 1). Mean daily temperature during egg laying was higher in 2008
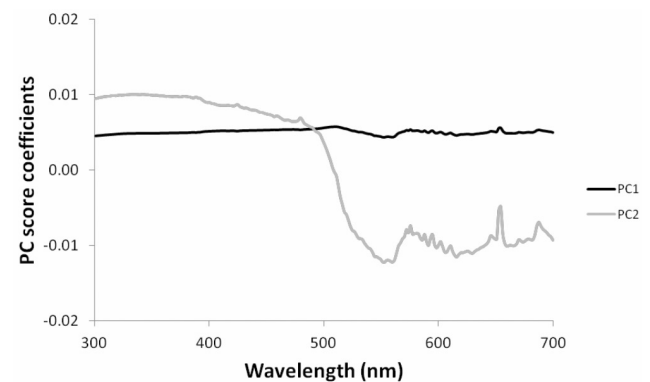

Fig. 3. PCA of great reed warbler eggshell reflectance spectra: PC1 and PC2 coefficients plotted against wavelength.

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compared to 2009 (Kruskal-Wallis $\chi^2 = 8.6$, df = 1, $P = 0.003$; Fig. 1). The mean daily amount of rainfall during egg laying also differed between the two study years (Kruskal-Wallis $\chi^2 = 17.6$, df = 1, $P < 0.0001$; Fig. 2).

Egg appearance

The first four principal components together explained 99.4 % of the variance in the reflectance spectra. PC1 explained 73.5 % of the total variance; it was flat and had positive values at all wavelengths, representing achromatic brightness (Fig. 3). PC2, PC3 and PC4 were not spectrally flat and thus represented chromatic aspects (hue and saturation) of egg colouration. PC2 explained 23.6 % of the total variance and had positive values at UV and blue wavelengths (Fig. 3). PC3 and PC4 together explained only 2.3 % of total variance and thus were not included in further analyses.

The best model explaining the variation in eggshell brightness (PC1) included year, temperature and egg-laying date. Clutches were darker in 2009 than in 2008, eggshell brightness increased with increasing temperature at laying and decreased throughout the season (Table 1).

The two top candidate models that best explained ($\Delta_i \leq 2$) the variation in PC2 scores (UV-blue) contained year, laying date and the average amount of rainfall during egg laying (Table 1). Model averaged parameter estimates of these two models showed that clutches in 2008 had higher PC2 than those in 2009 and eggs laid later in the season had lower PC2 (Table 1). The amount of precipitation, however, was not important as its 95 % CI included zero (Table 1).

Discussion

Both temperature and rainfall differed between the two study years and within each breeding season. Such variability in weather conditions among and within particular years offered us an ideal opportunity to test the hypothesis that spring meteorological conditions affect bird egg pigmentation (Avilés et al. 2007).

The models showed that mean temperature during egg laying has an effect on eggshell brightness (PC1), which increased with increasing temperature at laying. Weather did not affect UV-blue colouration of the eggs (PC2). In a between-year comparison, our findings are opposite to Avilés et al. (2007) who found that Eurasian reed warbler eggs tended to be bluer and greener in years with lower spring temperatures and had higher brightness in wetter years. By contrast, our study found that great reed warbler eggs reflected less in the UV and blue part of the spectrum (PC2) and were darker (PC1) in the colder year of our study. In addition, we found no effect of precipitation on great reed warbler egg colouration. This discrepancy in the results of the two studies is hard to explain. One possibility is that the role of environmental factors in egg colouration is species- and site-specific. The evidence for environmental effects on egg colouration remains controversial, since e.g. Gosler et al. (2000) and Mahler et al. (2008) found lack of consistent environmental effects on eggshell patterning.

It is interesting to note that PC2 also decreased with the progress of the breeding season. This variable could be affected by the age of breeding females, since older females lay earlier in the season than younger females (our unpublished data). An opposite relationship is typical of eggshell brightness (PC1), i.e. eggs laid late in the season were darker (this study, own unpublished data). However, at least for 2008, the temperature also increased as the breeding season progressed (Fig. 2A) and it is therefore difficult to separate the possibly inter-correlated factors of timing of breeding, temperature and female age.

In contrast to our finding, Hargitai et al. (2008) reported that blue-green showed seasonal increase in collared

Table 1. Results of model selection predicting PC1 and PC2 explaining great reed warbler colour clutch characteristics based on AIC$_c$. Model summary for PC1 shows the only candidate model, for PC2 model averaged estimates ± unconditional SE and 95% confidence intervals (CI) for each term across two top candidate models ($\Delta_i \leq 2$) are presented.

Dependent variable	Predictor	Estimate ± SE	Lower CI	Upper CI
PC1 (brigthness)	laying date	−0.009 ± 0.003	−0.003	−0.015
	temperature	0.024 ± 0.011	0.045	0.003
	year	−0.284 ± 0.064	−0.159	−0.410
PC2 (UV-blue)	laying date	−0.008 ± 0.004	−0.015	−0.0008
	rainfall	−0.004 ± 0.007	−0.018	0.010
	year	−0.368 ± 0.102	−0.568	−0.168

flycatcher eggs. The discrepancy between their and our study could be explained by diet differences, as collared flycatchers feed on different insect taxa that inhabit different habitats than great reed warblers. The effect of diet on blue-green eggshell colouration was experimentally confirmed by Moreno et al. (2006), who found that food-supplemented pied flycatcher females laid heavier and more intensely blue-green eggs than females that were not supplemented. To determine whether there is a direct effect of food on egg appearance in the great reed warbler, a thorough combination of diet analysis and experimental food supplementation is needed. However, great reed warblers feed on a large variety of different taxa ranging from insects to molluscs (Cramp 1992) and the relative effects of different dietary components on eggshell colouration are not known. Avilés et al. (2007, p. 476) hypothesised: "If eggshell colouration is related to the female condition at laying and/or indirectly dependent on food availability, we predicted associations between environmental conditions and egg colouration across the years." However, it has not yet been confirmed that weather affects the availability of all potential prey items in the same way. Molluscs and aquatic invertebrates, for example, may not be as dependent on temperature as terrestrial invertebrates are.

Apart from diet-mediated effects, it is also possible that the differences in eggshell colouration are influenced by thermoregulatory and metabolic processes that have nothing to do with diet. In a previous study, we found that great reed warbler females had low to moderate levels of repeatability in eggshell colouration (Honza et al. 2012). This further confirms that the effect of environmental or female intrinsic effects in some species may be quite significant. If this holds for more bird species, this would have important implications for the hypotheses trying to explain the variation in eggshell colouration. Most of these hypotheses focus on ultimate adaptive explanations, yet our results suggest that environmental variance should not be underestimated. It seems that at least part of the variance observed may simply reflect proximate conditions and may not necessarily be adaptive. We encourage further testing of the environmental hypothesis in other species breeding in variable habitats. Carefully designed experiments with supplemented individuals both in captivity and under natural conditions will reveal possible effects of diet on egg appearance.

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