Ethology Ecology & Evolution

Is UV and blue-green egg colouration a signal in cavity-nesting birds?

E. Węgrzyn[a]; K. Leniowski[b]; I. Rykowska[c]; W. Wasiak[c]
[a] Department of Zoology, University of Rzeszow, Rzeszow, Poland [b] Department of Avian Biology and Ecology, Adam Mickiewicz University, Poznań, Poland [c] Department of Analytical Chemistry, Faculty of Chemistry, Adam Mickiewicz University, Poznań, Poland

PLEASE SCROLL DOWN FOR ARTICLE

Taylor & Francis
Taylor & Francis Group

Is UV and blue-green egg colouration a signal in cavity-nesting birds?

E. Węgrzyn [1,4], K. Leniowski [2], I. Rykowska [3] and W. Wasiak [3]

[1] *Department of Zoology, University of Rzeszow, Rejtana 16c, 35-959 Rzeszow, Poland*
[2] *Department of Avian Biology and Ecology, Adam Mickiewicz University, Umultowska 89, 61-614 Poznań, Poland*
[3] *Department of Analytical Chemistry, Faculty of Chemistry, Adam Mickiewicz University, Grunwaldzka 6, 60-780 Poznań, Poland*

We analysed the eggs of the European starling *Sturnus vulgaris* for variability of UV and blue-green colouration. We noted the calcium deposition in eggshells, egg measurements, light conditions in nests and starling eye properties. Egg colouration shows high within-clutch similarity. Intensity of the blue-green egg colour neither depends on Ca deposition in the eggshell nor correlates with egg measurements, clutch size and laying date. This suggests that the intensity of the blue-green colour of starling eggs does not reflect female condition, which is reported to affect egg and clutch parameters. Low light intensity in cavities enables only mesopic vision, which is characterised by degraded visual acuity and colour sense, as well as Purkinje shift. As a consequence, the probability of precise assessment of the differences in eggs colour by starling males, crucial for the signalling hypothesis (MORENO & OSORNO 2003), is not high. On the other hand, irrespective of its intensity, blue-green is the last colour visible under decreased illumination. Thus such coloured eggs may attract higher parental attention in dark cavities. We also demonstrated a strong negative correlation between intensity of blue-green and UV colouration of starling eggs, as well as constrained sensitivity of the starling eye to the range of eggs' maximum UV reflectance. Our study indicates that it is blue-green, not the UV component of the egg's spectral characteristics, which seems to increase the perception of eggs by adults in dark cavities. We suggest that it might have been the most important pressure towards evolution of blue-green colouration of eggs in cavity-nesting birds.

KEY WORDS: European starling *Sturnus vulgaris*, UV and blue-green egg colouration, mesopic vision, birds' visual perception.

[4] Corresponding author: Ewa Węgrzyn, [current address] Department of Behavioural Ecology, Adam Mickiewicz University, Umultowska 89, 61-614 Poznań, Poland (E-mail: wegrzyn@amu.edu.pl).

INTRODUCTION

The traditional explanation of egg colouration in birds is related to selective pressures imposed by nest predators or brood parasites (UNDERWOOD & SEALLY 2002). More cryptic eggs help to avoid detection by predators in ground-nesting birds (CASTILLA et al. 2007; WESTMORELAND 2009) while higher within- than between-clutch egg similarity may enhance recognition of parasitic eggs by hosts (SOLER & MØLLER 1996; STOKKE et al. 1999). Another hypothesis is based on the mechanical properties of protoporphyrins, which in the form of the reddish spots compensate for reduced eggshell strength due to calcium deficiency (GOSLER et al. 2005). The function of UV reflectance of the eggs was proposed by AVILÉS et al. (2006a). The hypothesis assumes that UV provides detectability of eggs for incubating parents, which may enhance hatching success because detectability may play a main role in the movement associated with egg turning or settling onto the eggs. An explanation for blue-green egg colours was offered by MORENO & OSORNO (2003). They suggested that blue-green egg colouration is associated with the female condition and represents a signal of female quality that induces a higher allocation of male investment in parental care (but see REYNOLDS et al. 2009). According to the authors the link between the egg colour and the female condition is the pigment biliverdin – a potent antioxidant (KAUR et al. 2003), whose deposition in the eggshell may signal the antioxidant capacity of the female. The oxidative stress in females may reach an especially high level during the laying period due to progesterone action (VON SHANTZ et al. 1999). This would provide even better opportunity to determine female condition on the basis of the amount of biliverdin deposited on their eggshells.

Each of the hypotheses explaining different functions of egg colouration in birds has received some comparative, empirical or experimental support.

Starlings and their eggs

UV reach and blue-green eggs of the European starling *Sturnus vulgaris* theoretically may undergo at least a few different pressures, such as intraspecific nest parasitism, detectability of eggs by parents and sexual selection in the meaning of female quality advertisement. The two latter hypotheses were tested on the European starling or the closely related species the spotless starling, *Sturnus unicolor*. The study by AVILÉS et al. (2006a) provided some support for the view that UV egg colouration in starlings evolved to enhance egg detectability by parents in dim cavities. The intensity of blue-green egg colour was reported to reflect female quality (MORENO et al. 2006) and affect male preferences (HANLEY et al. 2008; SOLER et al. 2008); thus it was assumed to be a post-mating signal of females' quality.

However, before drawing conclusions on the adaptive or signalling function of a given feature, it is very important to check whether the interaction between the supposed selective pressure and the shaped feature is, at least theoretically, possible. Doing it in other ways involves the risk of finding connections which, in fact, are not there. Although it seems obvious, many studies lack this fundamental approach. Any assumptions on the function of egg colouration without taking into consideration light conditions in the nests and the perceptual characteristics of the bird eye seem highly questionable, the more so because a lot of such data is available in literature.

Does light make a difference?

Up-to-date studies supporting signalling function of blue-green egg colouration in birds have been conducted on cavity-nesting species, such as the Eastern bluebird, *Siaila sialix* (SIEFFERMAN et al. 2006), the pied flycatcher, *Ficedula hypoleuca* (MORALES et al. 2006) and the spotless starling (SOLER et al. 2008). Studies using operant conditioning indicate that birds are able to distinguish between very fine colour changes (HODOS 1993). These studies, however, relate to high light intensities enabling photopic (colour) vision. In poor light, which occurs in cavities, the probability of a switch to mesopic or scotopic (colourless) vision is very high. As with mammals, rods are the most sensitive to light and are the primary receptors for birds' vision in dim illumination (HART 2001). However, the transition from photopic (cone based) to scotopic (rod based) vision affects resolution, contrast sensitivity and colour perception (SHIN et al. 2004; ELOHOLMA 2005; STOCKMAN & SHARPE 2006). Consequently, ability to distinguish colours decreases rapidly below a luminance of 10 cd/m^2 (mesopic vision) and vision becomes monochromatic (scotopic) below 0.01 cd/m^2. Maximum image brightness of the starling eye approximately equals that of the human eye (MARTIN 1986). The recent study by LIND & KELBER (2009) has shown that some birds lose colour vision even at light levels 5 to 20 times higher than humans. Also models of the perceptual physiology of birds suggest that the nest luminosity significantly affects the perception of chromatic contrasts between eggs (AVILÉS 2008). As the result, objective differences in egg colour proved by spectrophotometric measurements may be beyond the perceptual abilities of birds due to physiological constraints on the eyes operating in the poor light available in the nest. Given that starling males can observe the eggs only in nests, estimating perception of colours in poor light seems crucial before drawing conclusions on the signalling function of blue-green egg colouration in cavity nesters.

Can UV-sensitive eyes see UV reach eggs?

Near-UV comprises wavelengths from 300 to 400 nm. The difference between its upper and lower wavelengths is analogous to, for example, the differences between green and red colour (500 and 600 nm respectively). Therefore, any conclusions on the function of egg UV reflectance in birds should be preceded by the analyses and comparisons of the range in which eggs reflect UV and the range in which the bird eye is sensitive to UV radiation. The range of peak sensitivity of particular photoreceptors in birds is narrow, therefore the general fact that eggs reflect UV radiation and birds' eyes are UV-sensitive does not necessarily mean that birds can see UV reflectance of their eggs.

The trade-off between the pigment and the base

Another overlooked fact is that biliverdin is not the only factor that has the potential to affect the intensity of egg colour. The other obvious determinant of the colour is calcium, which constitutes the white base of the eggshell. However, the relation between calcium concentration in the eggshell and egg colour has never been tested.

The aims of our study

We evaluated colour variation in starling eggs and we analysed within- and between-clutch variability of egg colouration. Subsequently, we tested the relation between intensity of blue-green egg colour and calcium concentration in the eggshell as well as such parameters of egg quality as size and mass. Finally, we were interested in whether adult birds are capable of noticing the differences in UV and blue-green colours of their eggs. For this reason we compared data of egg reflectance to starling eye properties with reference to sensitivity of photoreceptors and light conditions in cavities and nest-boxes where the eggs can naturally be watched by parents.

METHODS

Study area and species

The study was conducted in a population of European starlings *Sturnus vulgaris* in Czeszewo forest, north-west Poland (N52°06.720 E17°29.870) during the breeding season of 2009 in nest boxes installed earlier the same year. The European starling is a sexually dimorphic species that lays nonspotted blue-greenish eggs (CRAMP 1998) with a small peak at the ultraviolet wavelength (Fig. 1). Nest-boxes were inspected every 3 days to minimise the risk of nest desertion by females. As a female lays one egg a day (KESSEL 1957), the date of laying could be precisely determined without disturbing birds every day. Clutches contained 4–6 eggs. The range of laying dates in our study was 5 days. We studied the relationship between egg colouration and calcium concentrations in 21 eggs randomly chosen from 21 different clutches. One egg was collected from each clutch for chemical analyses. In 16 of these clutches we studied within- and between-clutch egg similarity for both colour and shape (79 eggs). All studied eggs were weighed with an
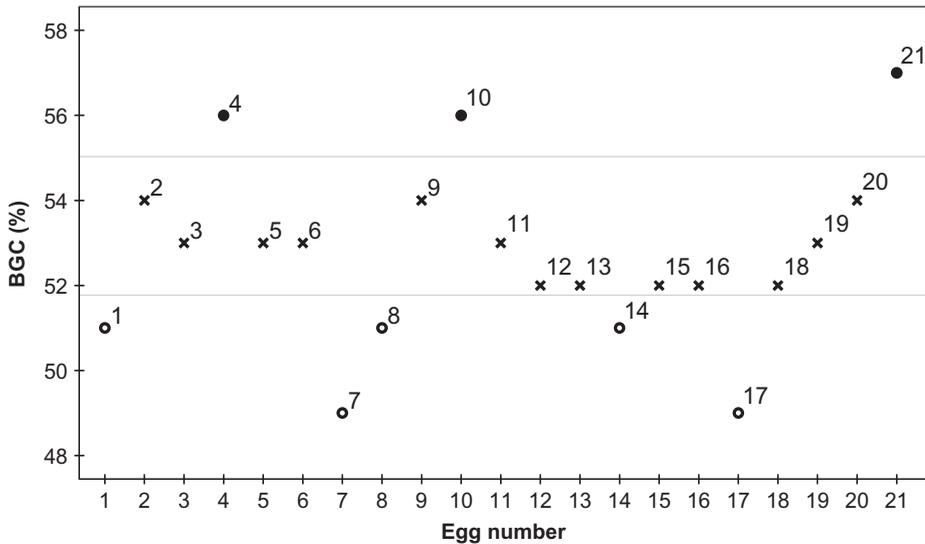

Fig. 1. — Classification of starling egg colours based on values of BGC measured with a spectrophotometer. The following colour categories were distinguished based on K-means cluster analysis with three cluster centers: ○, pale eggs; x, medium eggs; ●, dark eggs.

electronic balance to the nearest 0.01 g and measured with a slide caliper to the nearest 0.1 mm. We measured egg length and width (at its widest dimension). Egg colour was measured with a portable spectrophotometer. Measurements were taken within 2 days after the end of laying. To prevent nest desertion by parents, nest box entrances were always blocked during measurements. Eggs were returned to the nest within 20 min of removal. None of the nests was deserted in a subsequent period.

Chemical analysis

The concentration of Ca in the eggshell was assessed by the following method. The eggshells, with internal membrane removed, were weighed out in a Teflon bomb for mineralisation and then mixed with 5 ml of HNO_3 and 2 ml of 30% H_2O_2. After approximately 20 min the sample was mineralised in an MDS-2000 microwave oven. Next, the sample was quantatively transferred to a PE (polyethylene) flask (50 ml volume) and supplemented with redistilled water. Based on such prepared samples, the concentration levels of Ca were determined. The analysis was performed on an emission spectrometer with inductively coupled plasma VISTA-MPX (VARIAN).

The quantative analysis was performed by means of a calibration curve. With respect to the sample weight, the concentration per gram of Ca was determined.

Colour measurements

We classified egg colours using measurements of the reflectance spectra (300–700 nm) obtained from a Photon Control SPM-002 portable spectrophotometer connected to an SPL-1DH deuterium-halogen lamp, an SPA-200U reflectance probe and Spectrosoft Pro v. 2.3.1. software. The colour of each egg was measured on three randomly selected areas of the surface of the egg along the long egg axis (AVILÉS et al. 2006b). Each area was measured 5 times and the mean values were used in further analyses. The spectrophotometer covers the reflectance spectrum from 300 to 800 nm in intervals of 1 nm. Reflectances were measured with the probe placed at a constant distance and reaching the egg at 45°. Measurements were relative and referred to a standard white reference and to the dark, both calibrated before the measurement of each clutch. Reflectance, the term frequently used in our paper, is the proportion of light reflected at a given wavelength. Mean reflectance (of the entire spectrum or its range) was calculated as the sum of reflectances for each nanometer in the given spectral region divided by the number of nanometers. Blue-green chroma (BGC) was calculated as the proportion of total reflectance in the blue-green region of the spectrum (BGC = $Ref_{480–550nm}/Ref_{300–700nm}$, where $Ref_{480–550nm}$ is the sum of reflectances of wavelengths between 480 and 550 nm and $Ref_{300–700nm}$ is the sum of reflectances of wavelengths between 300 and 700 nm; SIEFFERMAN et al. 2006), which corresponds to the region of the lowest absorbance of biliverdin (FALCHUK et al. 2002) and because starling eggs reflect light maximally in it (Fig. 2). UV chroma (UVC) was calculated analogously to BGC as the proportion of total reflectance in the UV region of the spectrum (UVC = $Ref_{300–340nm}/Ref_{300–700nm}$) because starling eggs showed the highest UV reflectance between 300 and 340 nm.

Illumination measurements

Illumination at the bottom of a cavity and nest-box was measured with a TES-1332 portable photometer. A cavity, occupied earlier in the season by starlings, was cut out of the dead tree trunk and a starling nest-box was taken from the tree. Next, illumination inside each of them was measured with the hole directed towards the sun, against the sun and at a right angle to the sun. Measurements were taken at noon on a sunny and on a cloudy day. Each measurement was repeated 10 times and the mean was calculated. Measurements were taken with a starling dummy placed inside the cavity and nest-box to simulate the natural circumstances under which the eggs are watched by an adult bird.
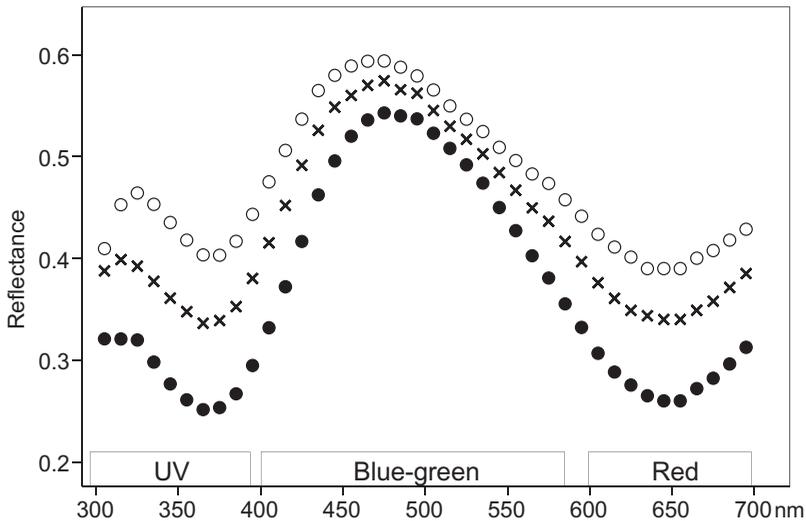
Fig. 2. — Reflectance values of pale (○), medium (x) and dark (●) eggs in the range 300–700 nm. Each plot is the mean of reflectances of three eggs from a given colour category.

Statistical analyses

Egg classification in the first part of the results was based on K-means cluster analysis with three cluster centers. Differences between eggs were tested with ANOVA, GLM and DFA. All analyses were conducted in SPSS.

RESULTS

Classification of egg colour intensity

We perceived the majority of collected starling eggs as intermediate and a few eggs as intensely blue or almost white. We entered BGC values of the 21 eggs collected from 21 different clutches in K-means cluster analysis with three cluster centers, which resulted in the classification of eggs presented in Fig. 1. BGC of the studied eggs varied from 49% to 57%. Eggs with a BGC below 52% ($n = 5$) were classified as pale. Medium eggs ($n = 13$) matched a BGC between 52 and 55%. Dark eggs ($n = 3$) were characterised by a BGC above 55%. Differences in BGC between the three categories were highly significant ($F = 48.647$, $P < 0.001$).

BGC and reflectance: are dark eggs more blue-green or less white?

In the previous section, Results, we showed that the BGC of dark eggs is significantly higher than the BGC of pale eggs. BGC is calculated as the proportion of the reflectance of the blue-green region and total reflectance of the egg. Thus different BGC values of dark and pale eggs may result from the differences in the reflectance

of the blue-green region as well as from the differences in the reflectance of the rest of the spectrum. If the higher BGC of dark eggs is due to an increased reflectance of the blue-green region at the same levels of reflectance of the rest of the spectrum, we should expect higher values of mean reflectance of the entire spectrum of dark eggs. The opposite should also hold true: lower values of the mean reflectance of dark eggs would indicate that the high BGC of dark eggs is caused not by increased reflectance in blue-green region, but by decreased reflectance outside this region. To see whether these differences are true, we conducted analyses of the mean reflectance of the entire spectrum of pale, medium and dark eggs. Dark eggs were characterised by lower over-all reflectance, especially in the spectrum outside the blue-green region (Fig. 2). The mean reflectance of dark eggs ($\bar{x} = 40\%$, SD $= 0.04$, $n = 4$) was lower than the mean reflectance of medium eggs ($\bar{x} = 44\%$, SD $= 0.04$, $n = 11$) and pale eggs ($\bar{x} = 49\%$, SD $= 0.04$, $n = 6$). The differences between the three categories of eggs were significant ($F = 5.44$, $P = 0.014$). Between-egg variation in mean reflectance (cv $= 11\%$) was much higher than variation in BGC (cv $= 4\%$). The above analysis shows that the differences in colour of dark and pale eggs result not only from their BGC but also from their brightness, represented by egg mean reflectance.

Detailed analysis of mean reflectance in the spectrum 300–400 nm (UV), 400–600 nm (blue-green) and 600–700 nm (red) revealed that the eggs differed almost twice as much in UV (cv $= 15\%$) and in the red spectrum (cv $= 15\%$) than in the blue-green region (cv $= 8\%$). BGC was significantly correlated with the mean reflectance of the entire spectrum ($R = -0.87$, $P < 0.001$) and the correlation was stronger for UV ($R = -0.89$, $P < 0.001$) and red ($R = -0.91$, $P < 0.001$) than for the blue-green spectrum ($R = -0.78$, $P < 0.001$, $n = 21$) when analysed separately. It is important to note that negative correlation between BGC and mean reflectance in the blue-green spectrum means that eggs of higher BGC (dark) were characterised by lower reflectance in the blue-green region than eggs of lower BGC (pale). Thus the dark eggs look more vivid not because they reflect more in the blue-green spectrum but because they reflect less outside this spectrum, which reduces the proportion of white content of the colour.

Within-clutch egg similarity in starlings

To test whether starling eggs are similar within the clutch we measured 79 eggs from 16 clutches. The parameters used in analyses are shown in Table 1. We found significant between-clutch differences analysing all parameters separately (Table 1) and in GLM ($F = 14.3$, $P < 0.001$). Correct classifications using DFA conducted on all presented parameters were 79% of original cases and 65% of cross-validated cases. BGC and mean reflectance explained 87% of the variance, but when using only colour parameters correct classifications decreased to 52% of original cases and 50% in a cross-validation procedure. Our analyses proved significant between-clutch differences in starling eggs, as well as a high probability of correct classification of eggs based on their colour and size.

Egg colours and calcium concentrations in the eggshells

As the white content of the spectrum was shown to affect egg BGC, we analysed Ca concentrations in eggshells to see whether this factor may affect egg colouration. However, we found no significant correlation between Ca concentration and egg mean

Table 1.

Between-clutch egg differences tested with ANOVA: $n = 79$ eggs and 16 clutches.

Egg parameter	F	P
Length	15.50	< 0.001
Width	4.41	< 0.001
Mean reflectance	13.34	< 0.001
BGC	19.00	< 0.001

reflectance (Spearman rank correlation: $r = 0.007$ $P = 0.5$, $n = 21$) or any other measure of egg colouration (BGC and UVC). Also, we did not find significant differences ($F = 1.128$, $P = 0.347$) in Ca concentration between pale ($\bar{x} = 376$, min = 353, max = 411, n = 5), medium ($\bar{x} = 367$, min = 351, max = 386, $n = 13$) or dark eggs ($\bar{x} = 367$, min = 352, max = 383, $n = 3$). Thus egg colouration in starlings does not depend on Ca deposition in the eggshell.

Relation between egg colour and egg/clutch quality

We did not find a significant correlation between colour parameters of the eggs (BGC, UVC and mean reflectance) and egg length, width, weight, laying date or clutch size (all $P > 0.05$, $n = 79$).

Light conditions in cavities and nest-boxes with reference to colour perception

In the previous section of the Results we demonstrated that starling clutches differed significantly in egg colouration. In this section we analyse whether these differences can be perceived by a receiver, for example a starling male. Colour perception and acuity of colour discrimination depends on illumination (ISHIDA 2002; LIND & KELBER 2009). At illumination levels above 10 cd/m^2 (64 lx) the cones primarily define the luminance or brightness of a light or surface. This is photopic vision, which enables perception and discrimination of colours. At illuminances below 0.01 cd/m^2 (0.06 lx) vision becomes rod-based and scotopic (colourless). Between these values vision is mesopic (mixed).

To determine what kind of the vision takes place in starling nests, we measured illumination both in the original cavity and in the nest-box. We conducted measurements with the hole directed towards the sun, against the sun and at a right angle to the sun. Measurements were taken at noon both on a sunny and on a cloudy day. Illumination values are presented in Table 2. Illumination of the eggs differed significantly depending on the hole direction both in cavities ($F = 590.458$, $P < 0.001$) and in nest-boxes ($F = 292$, $P < 0.001$). Illumination of the eggs in the cavity was lower than in the nest-box (t = 5.704, $P < 0.001$) irrespective of the direction of the hole. The light conditions both in a cavity and in a nest-box enabled only mesopic vision. The consequences of colour appearance in mesopic vision are presented in the Discussion section.

Table 2.

Illumination values in a cavity and a nest-box. Each value is a mean of 10 measurements. Photopic vision involves illumination greater than 10 lx, mesopic between 10 and 0.1 lx and scotopic below 0.1 lx.

Hole direction	Cavity				Nest-box			
	☼		☁		☼		☁	
	$\bar{x}$	SD	$\bar{x}$	SD	$\bar{x}$	SD	$\bar{x}$	SD
Towards the sun	7.7 lx	0.97	0.4 lx	0.01	40 lx	6.36	10 lx	2.22
Against the sun	0.2 lx	0.007	0.2 lx	0.007	5 lx	0.76	3 lx	0.48
At a right angle to the sun	0.2 lx	0.006	0.2 lx	0.007	5 lx	0.51	3 lx	0.52

Peak UV reflectance of eggs with reference to peak UV sensitivity of starling eyes

Aside from blue-green colouration, another spectral feature characteristic of starling eggs is UV reflectance with a peak between 310 and 330 nm (Figs 2–3). Eggs of different colours (pale, medium and dark) differ significantly in both UVC ($F = 6.69$, $P = 0.007$) and mean reflectance in the spectrum of 300–400 nm ($F = 7.64$, $P = 0.004$). To test whether the UV reflectance of starling eggs may be of functional relevance, we compared the range of maximum egg UV reflectance to starling eye properties (HART et al. 1998). Although starling eyes are UV-sensitive, their sensitivity is constrained. The spectral sensitivity of a cone photoreceptor is determined jointly by the spectral transmission of the oil droplet and ocular media (the lens and cornea) and the spectral absorbance of the visual pigment (KAWAMURO et al. 1997). The carotenoid-free T-type oil droplet associated with the UV-sensitive visual pigment in birds has no detectable absorption down to at least 330 nm. For this reason the short wavelength limit of photoreception is determined by the λ_{max} of the visual pigment and the spectral transmittance of the ocular media (HART 2001). In Fig. 3 we present the UV reflectance of starling eggs for wavelengths of 300–390 nm. On the same plot we marked the wavelength of UV peak sensitivity of starling cones ($\lambda_{max} = 360$ nm) and the wavelength

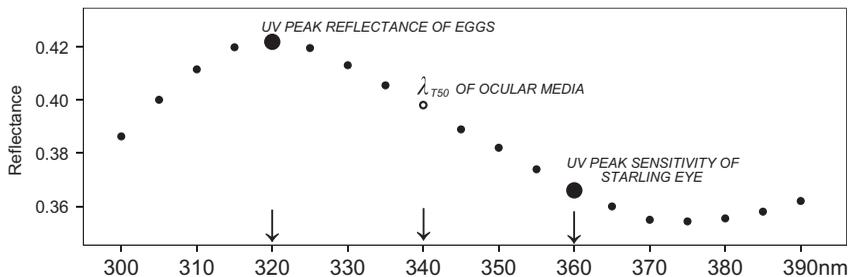Fig. 3. — UV reflectance of starling eggs (each value is the mean of the reflectance in a given wavelength of 21 eggs). On the same plot we marked: the wavelength of UV peak reflectance of eggs (320 nm), the wavelength of UV peak sensitivity of starling cones ($\lambda_{max} = 360$ nm) and the wavelength of 0.5 transmittance of ocular media ($\lambda_{T50} = 338$ nm). The two latter values are cited from HART et al. (1998).

of 0.5 transmittance of ocular media ($\lambda_{T50} = 338$ nm) (HART et al. 1998). The wavelength of UV peak sensitivity of the starling eye (360 nm) does not correspond to the eggs' maximum UV reflectance (320 nm). The mean reflectance of eggs in the wavelength of the eye UV peak sensitivity ($\bar{x} = 0.37$, SD $= 0.066$) is not significantly different ($F = 0.07$, $P = 0.795$) from the mean values of minimum UV reflectance in starling eggs ($\bar{x} = 0.365$, SD $= 0.065$), which corresponds to the wavelengths between 370 and 390 nm. Additionally, the wavelength of egg maximum UV reflectance (320 nm) is below the wavelength of 0.5 transmittance of ocular media (338 nm), which acts as a long-pass 'cut-off' filter. Comparison of spectral characteristics of eggs and the properties of starling eyes indicates that starlings are not sensitive to the UV reflectance of their eggs, at least in the range where the eggs reflect UV the most.

Relation between UV and blue-green colouration of starling eggs

The intensities of blue-green (BGC) and UV (UVC) colouration of starling eggs were strongly and negatively correlated ($R = -0.7$, $P < 0.001$) (Fig. 4). Mean UV reflectance of dark eggs ($\bar{x} = 23\%$, SD $= 0.008$, $n = 3$) was lower than mean UV reflectance of medium eggs ($\bar{x} = 30\%$, SD $= 0.023$, $n = 13$) and pale eggs ($\bar{x} = 36\%$, SD $= 0.037$, $n = 5$) (Fig. 1). The differences between the three categories of eggs were significant ($F = 22.730$, $P < 0.001$).

DISCUSSION

In the present paper we have analysed the variability of UV and blue-green colouration of starling eggs with reference to calcium deposition in eggshells, egg size and mass, light conditions in nests and starling eye properties. We discuss our results in following sections.
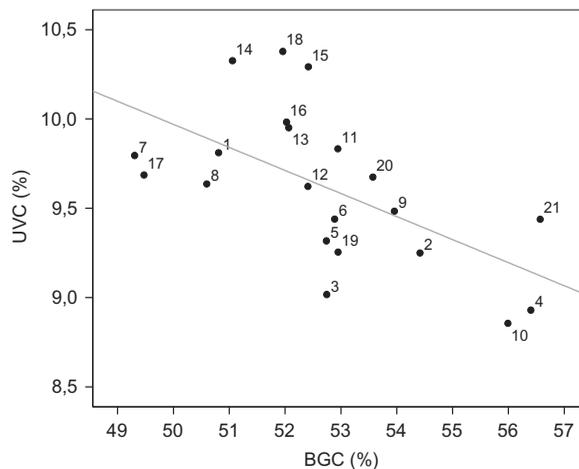

Fig. 4. — Relation between intensity of blue-green (BGC) and UV colouration (UVC) of starling eggs ($n = 21$). Eggs are numbered according to Fig. 1.

BGC and reflectance: are dark eggs more blue-green or less white?

We demonstrated that dark eggs of higher BGC were characterised by lower reflectance in the blue-green region than pale eggs. Although this may seem surprising, it can be easily explained by the spectral characteristics of biliverdin absorbance (FALCHUK et al. 2002), with characteristic peaks at 375 and 665 nm and low absorption at around 500 nm. Thus increasing concentration of biliverdin in an eggshell will slightly decrease the reflectance in the blue-green spectrum and greatly decrease the reflectance in the rest of the spectrum (in short and long wavelengths). This reduces the proportion of the white content of the colour, which looks more intense even though the reflectance in the blue-green region is slightly lower than in pale eggs.

Our analysis also shows that the differences in colour between dark and pale eggs results not only from their BGC but also from brightness, represented by egg mean reflectance. Darker eggs have lower mean reflectance, thus they are less bright than pale eggs. Decreased brightness, similarly to increased BGC, is likely to be a consequence of a higher concentration of the pigment (biliverdin), as it absorbs more light (the level of absorbance depends on pigment concentration). An important conclusion from this part of our study is that colour appearance results both from BGC (colour intensity) and mean reflectance (brightness). Eggs look darker due to a higher proportion of blue-green waves in the entire spectrum (BGC) and lower brightness (mean reflectance). The latter can be even more important in colour perception as between-egg variation in mean reflectance (cv = 11%) was considerably higher than variation in BGC (cv = 4%). This suggests that brightness may be worth including in analyses of egg colouration.

Within-clutch egg similarity in starlings

We found significant between-clutch differences both in colour parameters and in measurements of egg length and width. These results are in agreement with other studies on bird eggs (SMITH et al. 1995; CHRISTIANS 2002; MORENO et al. 2005; SIEFFERMAN et al. 2006). Within-clutch similarity is of relevance if the eggs are to signal female condition. However, higher between- than within-clutch differences in egg appearance may also result from heritable physiological characteristics of the females. In the village weaver, *Ploceus cucullatus*, for example, egg colour varies extensively from white to coloured (turquoise to emerald), and from faint to intense (COLLIAS 1993). COLLIAS (1993) reported that colour and the amount of spotting of the eggs of a given village weaver female were constant throughout her life, and the trait for type of egg colour is passed from mother to daughter. Similar results were obtained by GOSLER et al. (2000) in great tits, *Parus major*. On the other hand within-clutch egg similarity may constitute antiparasitic adaptation (DAVIES & BROOKE 1988; MØLLER & PETRIE 1991; LYON 2003; LYON & EADIE 2008; DOLORES et al. 2010). Intraspecific brood parasitism is quite frequent in starlings (EVANS 1988; LOMBARDO et al. 1989; FEARE 1991; PINXTEN et al. 1991a, 1991b; STOUFFER & POWER 1991; SANDELL & DIEMER 1999) with up to 37% of nests receiving parasitic eggs. Using DFA we demonstrated a high probability of correct classification of eggs based on their colour and size. Colour parameters (BGC and mean reflectance) explained 78% of the variance, which indicates that colour may be a clue used by females in recognising a parasitic egg. Starling females are reported not to remove parasitic eggs once they have started laying their own clutch but the evidence comes from a single study (STOUFFER et al.

1987). Thus between-clutch variation in colour and shape acting as antiparasitic adaptation cannot be fully rejected. On the other hand, a recent study by CASSEY et al. (2009) using a photoreceptor noise-limited colour opponent model of avian perception and data for spectral sensitivities of birds' eyes demonstrated that the studied species have a rather low ability to discriminate between the shell colouration of the eggs from different clutches (but see CASSEY et al. 2008a). We suggest that there is a need for further experimental studies testing whether starling females make use of the difference in appearance between parasitic eggs and their own ones.

Irrespective of its function, high within-clutch similarity of eggs in starlings allows for the possibility of considering an egg from a given clutch as a representative of the clutch.

Egg colours and calcium concentrations in the eggshells

We showed that egg colouration in starlings does not depend on Ca deposition in the eggshell. We were interested in the relation between calcium content in the eggshell and egg colouration for several reasons. First, as the colour results from the pigments, a higher concentration of Ca, which makes up the white colour of the eggs, may make the eggs paler. However, calcium deposition in eggshells may be condition-dependent, as is biliverdin deposition (LÓPEZ-RULL et al. 2008). From this point of view females of higher quality may lay eggs richer in both Ca and biliverdin, thus they may not differ in colour from the eggs of lower Ca concentration. This may explain the lack of the relation between egg colour and calcium content, but without data about biliverdin content it remains uncertain. Secondly, our interest in the relationship between calcium concentration and egg spectral characteristics emerged from the fact that the reflectance of an object, and consequently its colour, depends not only on the pigments but also on the surface structure. Smooth surfaces reflect more light than rough ones, thus glossiness may affect spectral characteristics by increasing overall reflectance of the object and thus making it brighter. Calcium content is one of the most important factors affecting eggshell structure in birds (BURLEY & VADEHRA 1989) so we tested the relation between Ca concentration in eggshells and mean reflectance of the eggs. Negative results of our analyses indicate that even if egg reflectance is affected by the differences in eggshell glossiness, this is not caused by structural differences of eggshells based on calcium content.

Relation between egg colour and egg/clutch quality

Surprisingly, although it seems basic, the relation between egg colour and egg biometry and/or laying date was rarely tested in the studies on blue-green colouration of eggs. There is a broad evidence that females of better condition lay larger (PARKER 2002) and heavier (STYRSKY et al. 1999) eggs. They also start laying earlier (WINKLER & ALLEN 1995) and produce more eggs (JONES & WARD 1976; FOGDEN & FOGDEN 1979; WINKLER & ALLEN 1995). Nestlings from larger eggs and earlier clutches survive better (SMITH & BRUUN 1998; ANDERSON & ALISAUSKAS 2001; PELAYO & CLARK 2003). In the light of the cited studies we may expect a correlation between egg colour and the studied parameters of the egg and clutch, if the colour is to reflect female or nestling quality. Contrary to this expectation, the intensity of the blue-green colour of starling eggs in our study does not correlate with egg size and mass as well as clutch size or laying date.

Similar results were obtained by CASSEY et al. (2008b) in a study on blue-green egg colouration of *Turdus* thrushes.

Our result does not support the hypothesis of MORENO & OSORNO (2003), who proposed that the intensity of blue-green egg colouration represents a signal of female quality. According to their hypothesis, biliverdin deposition in eggshell is costly for the female, as it is a potent antioxidant. However, we suggest that the cost of biliverdin deposition may be minor for starling females as their eggs are generally coloured quite subtly, which seems to involve low pigment concentrations. Even eggs considered as the darkest are much paler than, for example, the turquoise eggs of song thrushes. In the case of eggs of the song thrushes, close relatives of starlings (SIBLEY & AHLQUIST 1990), females have the capability to deposit much higher amounts of biliverdin in their eggs than the biliverdin present in starling eggs without adverse effects on their condition. The relatively low concentrations of biliverdin deposited in eggs by starling females may not be costly and dependent on female quality. An important, but often overlooked, fact is that biliverdin is formed during the process of heme degradation (YAMASHITA et al. 2004), so it is produced continuously. Being an endogenous waste product, biliverdin does not seem to be limited or costly in production. In some species, such as the crow tit (*Paradoxornis webbiana*), females have two colour types (blue and white) of eggs, but no nests contain mixed blue and white eggs. Egg colour is specific to the female and the breeding success of females laying blue and white eggs does not differ (KIM et al. 1995). Assuming that biliverdin deposition is costly and blue eggs are not advantageous in this species we should expect a decrease in blue pigmentation. Contrary to this expectation the ratio of blue clutches is higher or equal to white ones, depending on the population. Another study on Chinese domesticated chickens demonstrated that biliverdin deposited in eggshells is not derived from erythrocytes but synthesised in shell gland epithelial cells (ZHAO et al. 2006). The study showed no significant difference in biliverdin concentration in the blood of hens laying blue-shelled and of hens laying brown-shelled eggs. Thus, at least in the studied species, biliverdin deposition in eggs did not affect the antioxidant capacities of laying females. This questions the possible trade-off between female condition and intensity of blue-green egg colouration.

Light conditions in cavities and nest-boxes with reference to colour perception

Illuminance measurements indicate that light conditions enabled only mesopic vision in a cavity and a nest-box. We restrict our discussion to cavity illuminations as colouration of starling eggs evolved in cavities not in nest-boxes. Our measurements were conducted at noon when the light intensity is the highest, thus it is probable that at different times of day vision in cavities may be even scotopic. In mesopic vision the rod and cone outputs are pooled in shared nerve pathways. The rods strongly affect colour perception and contrast sensitivity by mixing with or tinting the colour responses of the still active cones. Mesopic vision is characterised by degraded visual acuity (ELOHOLMA 2005) and colour sense (SHIN et al. 2004). Peak sensitivity of rods is around 505 nm, which results in the shift of the photopic luminosity curve toward shorter waves (AL-AZZAWI 2006). Bright yellow, ochre and umber fuse into a single greyish tan, greens and blues appear as a single 'blue-green' colour, and reds become a warm, dark grey. This effect is called the Purkinje shift (PURKINJE 1819). In starlings, like most other vertebrates with a duplex retina, the Purkinje shift represents a transition from cone- to rod-mediated vision (DALLAND 1958). Although we lack an experimental approach, the

values of illumination in a cavity together with the characteristics of mesopic vision clearly indicate that the probability of precise assessment of the differences in egg colour by starling males is not high. Additionally, illumination inside the cavity changes depending on the weather and the position of the sun. This is very likely to affect colour perception by birds, i.e. the same eggs may look quite different under different illuminations. Change of colour appearance resulting from different light intensities in dim illumination was experimentally studied with human observers (SHIN et al. 2004). Perceived chroma of bluish and greenish colours reduced continuously with a decrease of illuminance level until 1 lx. This means that the same colour seemed less intense under lower illumination. Although the test was performed on humans, mesopic vision with characteristic peak sensitivity of rods around 500 nm is reported also in birds (MAIER 1992). Thus the changes of colour appearance resulting from different light intensities in dim illumination reported by SHIN et al. (2004) are very likely to apply to starlings and other cavity-nesting birds which perform visual tasks at low light intensity. We may expect that a proper assessment of egg colouration by a starling male under changeable illumination would involve correction for light intensity at the moment of watching the eggs, which seems highly speculative.

Another consequence of the Purkinje shift is the fact that 'blue-green' is the last visible colour under decreased illumination. This results from the fact that at low light intensities the spectral sensitivity of the eye is determined primarily by the spectral absorption properties of the rod visual pigment. The visual pigment in avian rods, similarly to all vertebrates, is rhodopsin and in starlings it has a peak sensitivity around 506 nm (blue-green). Studies of starling visual perception, both using behavioural data (DALLAND 1958) and analysis of rod visual pigment spectral absorption (HART et al. 1998) demonstrate that in dim illumination starling eyes are most sensitive to wavelengths between 440 and 560 nm (HART 2001). As visible in Fig. 2, maximum reflectance of starling eggs corresponds perfectly with the range of maximum rod sensitivity. We suggest that blue-green egg colouration in cavity-nesting birds might have evolved due to the visual perception characteristics of the parents' eyes, as wavelengths around 506 nm stimulate rods the most. This is also true in scotopic vision, where blue-green objects are perceived as brighter than object of other colours. If the illumination is reduced to below cone threshold, colour vision will disappear. Because the rods are more sensitive to short wavelengths than to long ones, blue colours will appear brighter, albeit colourless. Egg conspicuousness may play an important role during egg incubation but it may also affect other parental behaviours. Starling males are reported to adjust their parental investment to clutch size (KOMDEUR et al. 2002). It seems to be in the female's best interest to lay eggs that attract higher parental attention in dark cavities. From this point of view the differences in the intensity of egg colouration may be less important than the fact that all eggs are coloured in a way that stimulates starling photoreceptors the most under poor illumination, i.e. they reflect wavelengths between 440 and 560 nm.

Apart from degraded visual acuity and colour sense in mesopic vision, another problem faced by a male trying to asses egg colouration in a cavity arises from the fact that any visual task involves prior dark adaptation. When entering a dark environment, initially blackness is seen because the cones cease functioning in low-intensity light. Also, all the rod pigments have been bleached out due to the bright light and the rods are initially nonfunctional. Once in the dark, rhodopsin regenerates and the sensitivity of the retina increases over time. In diurnal birds, the initial stage of this process, when sensitivity of an eye increases rapidly, takes about 16 min. A further 25 min is

needed to completely accomplish the adaptation (WOLF 1945). We demonstrated that light available in a cavity provides dim illumination, thus dark adaptation is unavoidable when a male enters a cavity from a relatively bright environment. Interestingly, dark adaptation takes a longer time in the best-illuminated cavities directed towards the sun, due to increased levels of pre-adapting luminances acting on the bird's eye. Thus the assessment of egg colour, even if possible under dim illumination in a cavity, takes longer than a few minutes. Starling males participate in incubation (SMITH et al. 1995; KOMDEUR et al. 2002), therefore they have the possibility of watching their eggs following dark adaptation. However, they lack such a possibility when it comes to observing the clutches of other pairs. The latter seems to be important when categorising their own eggs as pale or dark, as colour intensity assessment is impossible without a reference. Although the colour reference in birds can come from the individual's memory (HODOS 1993), this would involve at least several breeding seasons. As the result, potential receivers of the signal of female quality, in the form of the intensity of blue-green egg colouration, would be restricted to old males only. This, in turn, is likely to decrease evolutionary pressure on quality signalling through egg colour, as the investment would not be rewarding for the females paired with younger males.

To sum up, poor illumination in cavities, where the colouration of starling eggs evolved, is not likely to favour the evolution of signalling through slight differences in colour intensity. The ability to perceive such differences by the receiver is necessary to promote the described evolution; however, it seems highly questionable whether starlings can discriminate colour chromas in mesopic vision. This assumption does not support the hypothesis of MORENO & OSORNO (2003), who proposed that the intensity of blue-green egg colouration represents a signal of female quality. On the other hand, irrespective of its intensity, blue-green colour seems to attract higher parental attention in dark cavities as their eyes have maximum sensitivity for blue-green colour in mesopic vision. This may be sufficient to promote evolution of biliverdin deposition in eggs. We propose this as an alternative hypothesis of the evolution and function of blue-green egg colouration. The possible correlation between the pigment amount in the eggshell and female condition found in some studies (SIEFFERMAN et al. 2006; LÓPEZ-RULL et al. 2008; SOLER et al. 2008) may be a by-product of such evolution, instead of its main target.

Peak UV reflectance of eggs with reference to peak UV sensitivity of starling eyes

Comparison of spectral characteristics of eggs and the properties of starling eyes indicates that starlings are not sensitive to UV reflectance of their eggs in the range where the eggs reflect UV the most. If UV reflectance of eggs evolved to increase egg detectability as proposed by AVILÉS et al. (2006a) we might expect its maximum to correspond with the wavelength of the UV peak sensitivity in the starling eye. Fig. 3 clearly demonstrates that this is not the case. Additionally, starling ocular media reduce the fraction of UV wavelengths that reach the retina, to those longer than 338 nm. As the perception of UV in the range of egg maximum UV reflectance in starling is constrained, we suggest that UV egg colouration did not evolve due to parental preferences towards UV-richer eggs. Interestingly, UV reflectance of starlings' nestling flanges, which was proved to play an important role in parent–offspring communication in starlings, has its maximum at about 360 nm (SOLER et al. 2007) and it corresponds perfectly with the wavelength of UV peak sensitivity in the starling eye. This clearly shows that the

evolution of signalling results in a close match of the signal and receptor properties. Such a relationship cannot be observed between the UV component of starling eggs and photoreceptor sensitivity, questioning the significance of UV reflectance of starling eggs. However, our results are observational and as such they do not provide evidence that starlings are blind to the UV reflectance of their eggs. We suggest that experiments testing the range of UV sensitivity of the starling eye are needed to fully reject the hypothesis of AVILÉS et al. (2006a).

Relation between UV and blue-green colouration of starling eggs

We demonstrated a strong negative correlation between the intensity of blue-green and UV colouration of starling eggs. The most likely explanation of such a pattern of egg colouration emerges from the biliverdin spectral characteristics with high absorbance in the UV region (FALCHUK et al. 2002). Higher biliverdin deposition in the eggshells increases intensity of blue-green colouration (BGC) but at the same time reduces UV reflectance, as more short wavelengths are absorbed. This, together with the limited sensitivity of the starling eye to the wavelengths of maximum egg reflectance in UV (300–340 nm), suggests that the UV reflectance of starling eggs does not result from any selective pressure leading to an adaptation or a signalling. Quite the contrary, UV may simply be the left-over of the original white colour of the eggs, which is presumed to be primeval (KILNER 2006). UV reflectance is characteristic of most white objects, including eggs, as they reflect all wavelengths equally. The presence of pigments that absorb short wavelengths, among others, causes objects of particular colours to lack UV reflectance to a degree which depends on pigment concentration. This seems to be the case with starling eggs, where the intensity of blue-green colouration is strongly and negatively correlated with UV reflectance. We suggest that evolution promoting blue-green egg colouration based on biliverdin deposition simultaneously acts against egg UV reflectance, which maximum anyway does not correspond to the wavelength of UV peak sensitivity of the starling eye, thus may be of little importance.

The combined assumption from our analyses is that it is the blue-green, not the UV component of the egg spectral characteristic, which seems to attract higher parental attention in dark cavities. We suggest that it might have been the most important pressure towards evolution of blue-green colouration of eggs in cavity-nesting birds.

REFERENCES

AL-AZZAWI A. 2006. Light and optics: principles and practices. *Florida: CRC Press*.

ANDERSON V.R. & ALISAUSKAS R.T. 2001. Egg size, body size, locomotion, and feeding performance in captive King Eider ducklings. *The Condor* 103: 195–199.

AVILÉS J.M. 2008. Egg colour mimicry in the common cuckoo *Cuculus canorus* as revealed by modelling host retinal function. *Proceedings of the Royal Society of London* (*B*) 275: 2345–2352.

AVILÉS J.M., SOLER J.J. & PÉREZ-CONTRERAS T. 2006a. Dark nests and egg colour in birds: a possible functional role of ultraviolet reflectance in egg detectability. *Proceedings of the Royal Society of London* (*B*) 273 (1603): 2821–2829.

AVILÉS J.M., SOLER J.J., PÉREZ-CONTRERAS T., SOLER M. & MØLLER A.P. 2006b. Ultraviolet reflectance of great spotted cuckoo eggs and egg discrimination by magpies. *Behavioral Ecology* 17 (2): 310–314.

BURLEY R.W. & VADEHRA D.V. 1989. The avian egg; chemistry and biology. *New York*: *John Wiley & Sons*.

CASSEY P., EWEN J.G., BLACKBURN T.M., HAUBER M.E., VOROBYEV M. & MARSHALL N.J. 2008b. Eggshell colour does not predict measures of maternal investment in eggs of *Turdus* thrushes. *Naturwissenschaften* 95: 713–721.

CASSEY P., EWEN J.G., MARSHALL N.J., VOROBYEV M., BLACKBURN T.M. & HAUBER M.E. 2009. Are avian eggshell colours effective intraspecific communication signals in the Muscicapoidea? A perceptual modelling approach. *Ibis* 151: 689–698.

CASSEY P., HONZA M., GRIM T. & HAUBER M.E. 2008a. The modelling of avian visual perception predicts behavioural rejection responses to foreign egg colours. *Biology Letters* 4: 515–517.

CASTILLA A.M., DHONDT A.A., DÍAZ-URIARTE R. & WESTMORELAND D. 2007. Predation in ground nesting birds: an experimental study using natural egg-color variation. *Avian Conservation and Ecology* 2 (1): 2. (Available at: http://www.ace-eco.org/vol2/iss1/art2/).

CHRISTIANS J.K. 2002. Avian egg size: variation within species and inflexibility within individuals. *Biological Reviews* 77: 1–26.

COLLIAS E.C. 1993. Inheritance of egg-color polymorphism in the village weaver *Ploceus cucullatus*. *Auk* 110: 683–692.

CRAMP S. (Ed.) 1998. The complete birds of the Western Palearctic on CD-ROM. *Oxford*: *Oxford University Press*.

DALLAND J.I. 1958. Spectral thresholds in the starling *Sturnus vulgaris*. *Ph.D. Thesis, Columbia University*.

DAVIES N.B. & BROOKE M. DE L. 1988. Cuckoos vs. reed warblers: adaptation and counter adaptations. *Animal Behaviour* 36: 262–284.

DOLORES M., LÓPEZ-DE-HIERRO G. & MORENO-RUEDA G. 2010. Egg-spot pattern rather than egg colour affects conspecific egg rejection in the house sparrow *Passer domesticus*. *Behavioral Ecology and Sociobiology* 64 (3): 317–324.

ELOHOLMA M. 2005. Development of visual performance based mesopic phoyometry. *Ph.D. Thesis, Helsinki University of Technology*.

EVANS P.G.H. 1988. Intraspecific nest parasitism in the European starling *Sturnus vulgaris*. *Animal Behaviour* 36: 1282–1294.

FALCHUK K.H., CONTIN J.M., DZIEDZIC T.S., FENG Z., FRENCH T.C., HEFFRON G.J. & MONTORZI M. 2002. A role for biliverdin IXa in dorsal axis development of *Xenopus laevis* embryos. *Proceedings of the National Academy of Sciences of the USA* 99: 251–256.

FEARE C.J. 1991. Intraspecific nest parasitism in starlings *Sturnus vulgaris*: effects of disturbance on laying females. *Ibis* 133: 75–79.

FOGDEN M.P. & FOGDEN P.M. 1979. The role of fat and protein reserves in the annual cycle of the grey-backed Camaroptera in Uganda (Aves: Sylvidae). *Journal of Zoology* 189: 233–258.

GOSLER A.G., BARNETT P.R. & REYNOLDS S.J. 2000. Inheritance and variation in eggshell patterning in the great tit *Parus major*. *Proceedings of the Royal Society of London* (*B*) 267: 2469–2473.

GOSLER A.G., HIGHAM J.P. & REYNOLDS S.J. 2005. Why are birds' eggs speckled? *Ecology Letters* 8: 1105–1113.

HANLEY D., HEIBER G. & DEARBORN D.C. 2008. Testing an assumption of the sexual-signalling hypothesis: does blue-green egg color reflect maternal antioxidant capacity? *The Condor* 110 (4): 767–771.

HART N.S. 2001. Variation in cone photoreceptor abundance and the visual ecology of birds. *Journal of Comparative Physiology* (*A*) 187: 685–698.

HART N.S., PARTRIDGE J.C. & CUTHILL I.C. 1998. Visual pigments, oil droplets and cone hotoreceptor distribution in the European starling (*Sturnus vulgaris*). *Journal of Experimental Biology* 201: 1433–1446.

HODOS W. 1993. The visual capabilities of birds, p. 6376. In: Zeigler H.P. & Bischof H.J., Eds. Vision, brain, and behavior in birds. *Cambridge*, *MA*: *MIT Press*.

ISHIDA T. 2002. Color identification data obtained from photopic to mesopic illuminance levels. *Color Research & Application* 27: 252–259.

JONES P.J. & WARD P. 1976. The level of reserve protein as the proximate factor controlling the timing of breeding and clutch-size in the red-billed quelea *Quelea quelea*. *Ibis* 118: 547–574.

KAUR H., HUGHES M.N., GREEN C.J., NAUGHTON P., FORESTI R. & MOTTERLINI R. 2003. Interaction of bilirubin and biliverdin with reactive nitrogen species. *FEBS Letters* 543: 113–119.

KAWAMURO K., IRIE T. & NAKAMURA T. 1997. Filtering effect of cone oil droplets in the P-III response spectra of Japanese quail. *Vision Research* 37: 2829–2834.

KESSEL B. 1957. A study of the breeding biology of the European starling (*Sturnus vulgaris* L.) in North America. *The American Midland Naturalist* 58: 257–331.

KILNER R.M. 2006. The evolution of egg colour and patterning in birds. *Biological Reviews* 81: 383–406.

KIM C.-H., YAMAGISHI S. & WON I.-O. 1995. Egg-color dimorphism and breeding success in the crow tit *Paradoxornis webbiana*. *The Auk* 112 (4): 831–839.

KOMDEUR J., WIERSMA P. & MAGRATH M. 2002. Paternal care and male mate-attraction effort in the European starling is adjusted to clutch size. *Proceedings of the Royal Society of London* (*B*) 269: 1253–1261.

LIND O., & KELBER A. 2009. The intensity threshold of colour vision in two species of parrot. *Journal of Exprimental Biology* 212: 3693–3699.

LOMBARDO M.P., POWER H.W., STOUFFER P.C., ROMAGNANO L.C. & HOFFENBERG A.S. 1989. Egg removal and intraspecific brood parasitism in the European starling *Sturnus vulgaris*. *Behavioral Ecology and Sociobiology* 24: 217–223.

LÓPEZ-RULL I., MIKSIK I. & GIL D. 2008. Egg pigmentation reflects female and egg quality in the spotless starling *Sturnus unicolor*. *Behavioral Ecology and Sociobiology* 62: 1877–1884.

LYON B.E. 2003. Egg recognition and counting reduce costs of avian conspecific brood parasitism. *Nature* 422: 495–499.

LYON B.E. & EADIE J.McA. 2008. Conspecific brood parasitism in birds: a life-history perspective. *The Annual Review of Ecology, Evolution, and Systematics* 39: 343–363.

MAIER E.J. 1992. Spectral sensitivities including the ultraviolet ofthe passeriform bird *Leothrix lutea*. *Journal of Comparative Physiology* (*A*) 170: 709–714.

MARTIN G.R. 1986. The eye of a passeriform bird, the European starling *Sturnus vulgaris*: eye movement amplitude, visual fields and schematic optics. *Journal of Comparative Physiology* (*A*) 159: 545–557.

MØLLER A.P. & PETRIE M. 1991. Evolution of intraspecific variability in bird's egg: is intraspecific nest parasitism the selective agent? *Proceedings of International Ornithological Congress* 20: 1041–1048.

MORALES J., SANZ J.J. & MORENO J. 2006. Egg color reflects the amount of yolk maternal antibodies and fledging success in a songbird. *Biology Letters* 2 (3): 334–336.

MORENO J., LOBATO E., MORALES J., MERINO S., TOMAS G., MARTINEZ-DE LA PUENTE J., SANZ J.J., MATEO R. & SOLER J.J. 2006. Experimental evidence that egg color indicates female condition at laying in a songbird. *Behavioral Ecology* 17: 651– 655.

MORENO J., MORALES J., LOBATO E., MERINO S., TOMAS G. & MARTINEZ-DE LA PUENTE J. 2005. Evidence for the signaling function of egg color in the pied flycatcher. *Behavioral Ecology* 16: 931–937.

MORENO J. & OSORNO J.L. 2003. Avian egg colour and sexual selection: does eggshell pigmentation reflect female condition and genetic quality? *Ecology Letters* 6: 803–806.

PARKER T.H. 2002. Maternal condition, reproductive investment, and offspring sex ratio in captive red junglefowl *Gallus gallus*. *The Auk* 119 (3): 840–845.

PELAYO J.T. & CLARK R.G. 2003. Consequences of egg size for offspring survival: a cross-fostering experiment in ruddy ducks *Oxyura jamaicensis*. *The Auk* 120 (2): 384–393.

PINXTEN R., EENS M. & VERHEYEN R.F. 1991a. Conspecific nest parasitism in the European starling. *Ardea* 79: 15–30.

PINXTEN R., EENS M. & VERHEYEN R.F. 1991b. Responses of male starlings to experimental intraspecific brood parasitism. *Animal Behaviour* 42: 1028–1030.

PURKINJE J. 1819. Beiträge zur Kenntnis des Sehens in subjectiver Hinsicht. *Prague: J.G. Calve*.

REYNOLDS S.J., MARTIN G.R. & CASSEY P. 2009. Is sexual selection blurring the functional significance of eggshell coloration hypotheses? *Animal Behaviour* 78: 209–215.

SANDELL M.I. & DIEMER M. 1999. Intraspecific brood parasitism: a strategy for floating females in the European starling. *Animal Behaviour* 57: 197–202.

SHIN J.C., MATSUKI N., YAGUCHI H. & SHIORI S. 2004. A color appearance model applicable in mesopic vision. *Optical Review* 11 (4): 272–278.

SIBLEY C.G. & AHLQUIST J.E. 1990. Phylogeny and classification of birds: a study in molecular evolution. *New Haven*: *Yale University Press*.

SIEFFERMAN L., NAVARA K.J. & HILL G.E. 2006. Egg coloration is correlated with female condition in eastern bluebirds. *Behavioral Ecology and Sociobiology* 59: 651–656.

SMITH H.G. & BRUUN M. 1998. The effect of egg size and habitat on starling nestling growth and survival. *Oecologia* 115: 59–63.

SMITH H.G., SANDELL M.I. & BRUUN M. 1995. Paternal care in the European starling *Sturnus vulgaris*: incubation. *Animal Behaviour* 50: 323–331.

SOLER J.J., AVILÉS J.M., CUERVO J.J. & PÉREZ-CONTRERAS T. 2007. Is the relation between colour and immune response mediated by nutritional condition in spotless starling nestlings? *Animal Behaviour* 74: 1139–1145.

SOLER J.J. & MØLLER A.P. 1996. A comparative analysis the evolution of variation in appearance of eggs European passerines in relation to brood parasitism. *Behavioral Ecology* 7: 89–94.

SOLER J.J., NAVARRO C., PÉREZ-CONTRERAS T., AVILÉS J. & CUERVO J.J. 2008. Sexually selected egg coloration in spotless starlings. *The American Naturalist* 171: 183–194.

STOCKMAN A. & SHARPE L.T. 2006. Into the twilight zone: the complexities of mesopic vision and luminous efficiency. *Ophthalmic and Physiological Optics* 26 (3): 225–239.

STOKKE B.G., MOKSNES A., ROSKAFT E., RUDOLFSEN G. & HONZA M. 1999. Rejection of artificial cuckoo *Cuculus canorus* eggs in relation to variation in among reed warblers *Acroceph alus scirpaceus*. *Proceedings of the Royal Society of London* (*B*) 266: 1483–1488.

STOUFFER P.C., KENNEDY E.D. & POWER H.W. 1987. Recognition and removal of intraspecific parasite eggs by starlings. *Animal Behaviour* 35: 1583–1584.

STOUFFER P.C. & POWER H.W. 1991. Brood parasitism by starlings experimentally forced to desert their nests. *Animal Behaviour* 41: 537–539.

STYRSKY J.D., ECKERLE K.P. & THOMPSON C.F. 1999. Fitness-related consequences of egg mass in nestling house wrens. *Proceedings of the Royal Society of London* (*B*) 266: 1253–1258.

UNDERWOOD T.J. & SEALY S.G. 2002. Adaptive significance of egg coloration, pp. 280–298. In: Deeming D.C., Ed. Avian incubation: behaviour, environment, and evolution. *Oxford*: *Oxford University Press*.

VON SCHANTZ T., BENSCH S., GRAHN M., HASSELQUIST D. & WITTZELL H. 1999. Good genes, oxidative stress and condition-dependent sexual signals. *Proceedings of the Royal Society of London* (*B*) 266: 1–12.

WESTMORELAND D. 2009. Evidence of selection for egg crypsis in conspicuous nests. *Journal of Field Ornithology* 79 (3): 263–268.

WINKLER D.W. & ALLEN P.E. 1995. Effects of handicapping on female condition and reproduction in tree swallows *Tachycineta bicolor*. *The Auk* 112 (3): 737–747.

WOLF E. 1945. Effect of exposure to ultra-violet light on subsequent dark adaptation. *Proceedings of the National Academy of Sciences of the USA* 31 (11): 349–355.

YAMASHITA K., MCDAID J., OLLINGER R., TSUI T.Y., BERBERAT P.O., USHEVA A., CSIZMADIA E., SMITH R.N., SOARES M.P. & BACH F.H. 2004. Biliverdin, a natural product of heme catabolism, induces tolerance to cardiac allografts. *FASEB Journal* 18: 765–767.

ZHAO R., XU G.-Y., LIU Z.-Z., LI J.-Y. & YANG N. 2006. A study on eggshell pigmentation: biliverdin in blue-shelled chickens. *Poultry Science* 85: 546–549.