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Sexual selection based on egg colour: physiological models and egg discrimination experiments in a cavity-nesting bird

Jesús M. Avilés · Juan J. Soler · Nathan S. Hart

Abstract It has been proposed that the blue-green bird egg colourations of many avian species may constitute a sexually selected female signal that males can use to modulate their parental investment. A fundamental untested assumption for the validation of this hypothesis is that males can accurately assess differences in the colour of eggs. A recent review suggests that this could be particularly problematic when egg clutches were located within a dimly lit nest cavity, due to limitations of the visual system in low light conditions. Here, we first used a photoreceptor noise-limited model of colour discrimination ability that accounts for visual performance under low light conditions to study whether a typical cavity-nesting passerine, the spotless starling *Sturnus unicolor*, can discriminate their eggs under the ambient illumination in their nest-holes. Secondly, we tested the validity of model predictions with behavioural data collected in two egg discrimination experiments performed in this species. Estimated egg detectability depended entirely on model assumptions about visual limitations linked to light intensity. Starlings would not be able to discriminate egg differences in their nests if the model was based on the assumption that light intensity limited detectability, whereas they could potentially perceive as different many possible pairwise clutch comparisons if the model assumption was that light intensity did not limit detectability. Results of behavioural experiments fitted the prediction of the visual model where light intensity did not limit detectability. Our results suggest that photoreceptor noise-limited colour models based on stimulation of single photoreceptors cannot, at present, be used to predict egg discrimination ability in spotless starlings under low light conditions. Future studies aiming to test egg discrimination constraints in the frame of the sexual selection hypothesis should therefore combine both modelling and behavioural experiments to determine which are the components of the models that produce the mismatch with the behavioural conditions.

Keywords Cavity nests · Light intensity · Blue eggs · Visual perception · Spotless starling · Sexual selection

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Introduction

Recently, Moreno and Osorno (2003) proposed that blue-green bird egg colourations may have evolved as signals of female quality that males can use to modulate their parental investment (sexual selection hypothesis; hereafter referred to as the SSH). The reasoning behind the SSH is that biliverdin, which is the pigment responsible for blue-green egg colouration in birds (Kennedy and Vevers 1976; Miksik et al. 1996), possesses strong antioxidant activities (Mcdonagh 2001; Kaur et al. 2003), and only those females with a high antioxidant capacity would be able to exaggerate the use of biliverdin as an eggshell pigment (Moreno and Osorno 2003). It follows that male birds might modulate their allocation of parental effort by using eggshell colouration as a reliable estimator of female phenotypic quality or condition

(i.e. attractiveness), according to the differential allocation hypothesis (Burley 1986; Sheldon 2000).

In a recent paper, Reynolds et al. (2009) reviewed the literature and searched for studies testing predictions emerging from the SSH. They concluded that "there are substantive reasons for questioning whether eggshell colouration can reliably signal heritable female fitness to another bird, especially in cavity-nesting species". Their main criticism of the SSH was derived from an obvious but untested assumption that it would be difficult for a bird to accurately assess differences in the colour of eggs that were located within a dimly lit nest cavity due to limitations of their visual system.

Beyond low luminosity constraining egg discrimination in hole-nesting birds, Cassey et al. (2009) used a model of avian visual perception (Vorobyev and Osorio 1998) to assess the potential for the SSH in 46 species of the superfamily *Muscicapoidea*. Based on model calculations alone, they questioned that, in the majority of the 46 species, birds would be able to discriminate between eggs in different conspecific clutches (Cassey et al. 2009). As acknowledged by the authors, however, model calculations were based on reflectance data of eggs that were collected almost a century ago and thus may have faded (e.g. Avilés et al. 2007). Interestingly, they also found that pairwise discriminability estimated by visual models in freshly collected clutches of open-nester common blackbirds *Turdus merula* and song thrushes *Turdus philomelos* was low (Cassey et al. 2009), which would indicate a major limitation for the functioning of the SSH for open-nester species too. Cassey et al. (2009) did not test predictions arising from their visual models about low egg-clutch discriminability with behavioural experiments.

Surprisingly, previous behavioural studies have shown that blackbirds have the potential to discriminate (i.e. reject) experimental model eggs that resemble real blackbird egg colours under the luminal conditions prevailing in their nests (Davies and Brooke 1989; Moskat et al. 2003). More importantly, a recent study assessing the discriminability of great tit *Parus major* eggs based on visual models found that egg detectability depended entirely on model assumptions of visual limitations (Lind and Kelber 2009a; Holveck et al. 2010). These results challenge the predictions of the avian visual model used by Cassey et al. (2009) and call for further investigations within this field before general trends and concepts on signalling potential of egg colouration can be disclosed.

The use of behavioural experiments to establish threshold values for discrimination is well-established in visual perception literature (reviewed in Kelber et al. 2003; Lind and Kelber 2009b). Combining behavioural tests on egg colour perception and visual models seems essential given growing evidence showing that birds still can discriminate

between visual stimuli that, according to visual colour modelling, would be non-discriminable (e.g. Holveck et al. 2010; Avilés et al. 2010).

In this study, we aim to test the validity of Reynolds et al.'s (2009) argument against the SSH using both models of avian visual ability and careful behavioural experiments of egg discrimination in a typical cavity-nesting passerine, the spotless starling *Sturnus unicolor*. Previous correlative and experimental evidence suggests that starlings are an ideal model species with which to test egg discrimination in the context of the SSH: Spotless starlings lay blue eggs (e.g. Soler et al. 2008; Fig. 1a), and egg blueness is a reliable predictor of biliverdin pigment content in the eggs of this species (López-Rull et al. 2008). Furthermore, males can modify their parental investment based on colouration of eggs, which was shown to be a female condition dependent trait in starlings (Soler et al. 2008). For another cavity-nesting species, the pied flycatchers *Ficedula hypoleuca*, Moreno et al. (2008) found evidence of parental favouritism in clutches provided with a 'supernormal' dummy blue egg, which suggests that females laying a supernormal coloured egg could potentially gain enhanced paternal contribution to reproduction. Thus, the SSH could work by males comparing eggshell colouration of either different clutches or perceiving intra-clutch variation in egg colour intensity.

Here, we first used a photoreceptor noise-limited colour opponent model similar to that used in Cassey et al. (2009) to study whether starlings can discriminate their eggs from the nesting material on which they are laid under the ambient illumination in their nest-holes. Secondly, we studied the variability in egg colouration (between and within clutches) as would be perceived in cavity environments (i.e. in dim light conditions) by potential receivers of the signal (i.e. male birds). In order for male starlings to assess the phenotypic fitness of different females and to allocate parental resources accordingly, the difference in eggshell colouration between clutches must be greater than the thresholds of visual discrimination of the male. Finally, we evaluated the potential of a spotless starling male to differentiate between the eggs of a single clutch.

In a second stage, we tested the predictions arising from avian visual models in the context of the SSH by reanalyzing previously published spectrophotometric data and comparing these results with the behavioural responses reported in two behavioural egg discrimination studies performed in spotless starlings (Avilés et al. 2006; Soler et al. 2008). In these two experiments, we measured behavioural responses of spotless starlings to changes in egg colouration that we quantified with a spectroradiometer. Here, we used recently published data on nest reflectance (Avilés et al. 2008), nest irradiance measures in starling nests, and incorporated published spectral sensitivity data on European starlings (Hart et al. 1998; Hart and Vorobyev
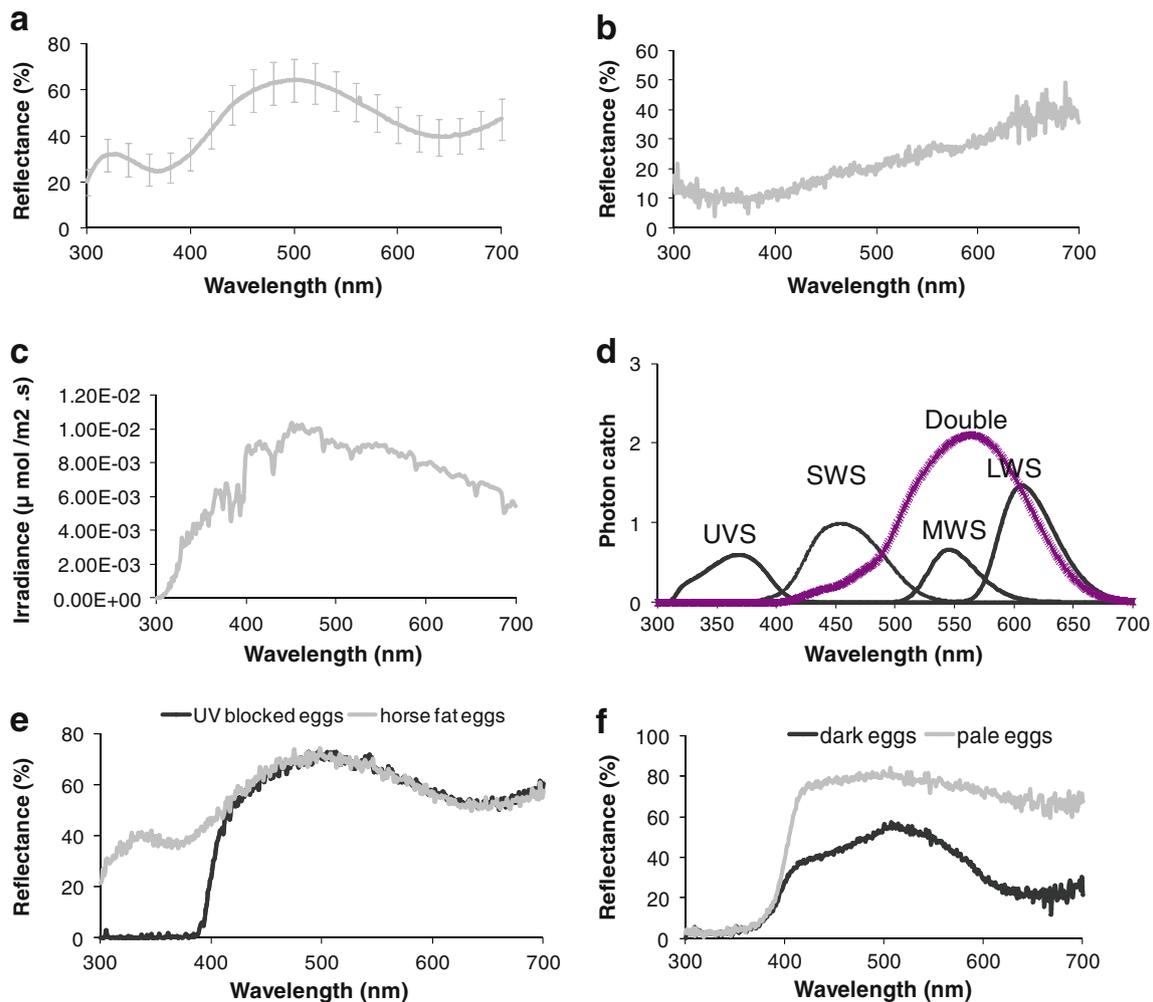
Fig. 1 a Reflectance spectra (mean±SD) of spotless starling eggs in Guadix (*N*=135 starling eggs at 31 occupied nest-boxes). **b** Reflectance spectra of nest material in spotless starling eggs in Guadix (*N*=3 nests). **c** Irradiance spectra of the nest light in nest-box cavities occupied by spotless starlings after Avilés et al. (2008). **d** Spectral sensitivity of single (used to model chromatic contrasts) and double (used to model achromatic contrasts) cones of European starling *Sturnus vulgaris* after accounting for ocular media light transmission properties after Hart et al. (1998) and Hart and Vorobyev (2005). **e** Average reflectance spectra of spotless starlings eggs when they were treated with horse fat containing or not containing UV-light blocker to experimentally study egg retrieval in relation to ultraviolet reflectance of the eggs after Avilés et al. (2006). **f** Average reflectance spectra of dark and pale blue eggs used to test the SSH in spotless starlings after Soler et al. (2008)

2005) that allow us to model visual perception of signal intensity emitted by spotless starling eggs in these experiments, thus facilitating a test of reliability of predictions emerging from avian perception models in cavity–nest environments.

Material and methods

The study population and data collection

The study was carried out in a spotless starling colony in Guadix, south-eastern Spain (37°18′N, 3°11′W), settled on *Quercus ruber* cork made nest-boxes (for a further description of the study area, see Soler et al. 2008). All nest-boxes in this study were in sunlight.

To model egg discriminability, we used reflectance spectra of 135 starling eggs at 31 occupied nest-boxes measured 2 days after the end of laying following the protocol described in Soler et al. (2008) during the breeding season of 2005. These nests compound a subset of the nests sampled in the Soler et al. (2008) study, where 2005 and 2006 reflectance data were used, to describe colouration of spotless starling eggs (see Fig. 1 in Soler et al. 2008). In short, reflectance spectra (300–700 nm) of all the eggs in a clutch were recorded with an Ocean Optics USB2000 spectrometer, a deuterium–halogen light (D2-W, mini), a bifurcated optical fiber (QR-400-7-UV-VIS), and the

OOIBase32 operating software (Ocean Optics, Inc., Dunedin, FL, USA). Reflectance was always measured with the probe placed at a constant distance and at an angle of 45° to the surface of the egg. Measurements were relative and referenced to a standard white reference (WS-2; Ocean Optics) and to the dark, which we calibrated before the measurement of each clutch.

Reflectance data of spotless starling nests were already measured in three nests in a previous comparative study on nestling detectability (Avilés et al. 2008). These nests compound a subset of those sampled in the Avilés et al. (2008) study to describe colouration of nest line material of species that build a nest cup mainly constituted by dry grass. In the laboratory, we measured the spectral reflectance of the nest lining with an Ocean Optics spectroradiometer, as described above. All measurements were made in a darkened room. For every collected nest, the material of the nest lining was disassembled and representative materials laid flat on a black table for measurements (for a further description, see Avilés et al. 2008). Ten readings were taken from every nest, and the average reflectance of a spotless starling nest was calculated on average values per nest (Fig. 1b).

Illumination at the spotless starling nest cavities was measured during the morning (09.00–11.00 AM), using an Ocean Optics USB2000 spectrometer with a cosine-corrected fiber-optic probe (P400-1-UV-VIS, Ocean Optics) with a 180° angle of acceptance and a measurement surface of 6 mm in diameter (CC-3-UV, Ocean Optics). The spectrometer was calibrated with light source of known colour temperature (LS-1-CAL; Ocean Optics). To get representative measurements of ambient irradiance at starling nest cavities, we gently introduced the probe from the entrance and collected ten irradiance readings at the bottom of nest-boxes (i.e. about 10–15 cm over the eggs) and pointing vertically towards the roof. The hand holding the optic fibre was, therefore, at the entrance of the nest-box blocking some of the light entering the cavity. Consequently, our measurements included the effect of a bird in the entrance to the cavity with its body blocking some of the light (see Reynolds et al. 2009). Average irradiance in typical nest-box cavities occupied by spotless starlings was calculated on average values of irradiance per nest (Fig. 1c). Total irradiance from 300 to 700 nm summed 2.9 μmol.

The model

We calculated egg discriminability from the perspective of a signal receiver using the colour opponency model of Vorobyev and Osorio (1998) developed for the tetrachromatic visual system of birds in its log form (Vorobyev et al. 1998) with Avicol software v3 (Gomez 2006). This model

successfully described thresholds for visual discrimination in birds (Vorobyev and Osorio 1998; Goldsmith and Butler 2005). Furthermore, the application of the model has been successful in predicting egg discrimination behaviour in birds under photopic conditions (Avilés 2008; Cassey et al. 2008; Avilés et al. 2010). This model establishes a colour distance ΔS which describes the colour contrasts between two eggs as:

$$
\begin{aligned}
\Delta S^2 = [&(e_1 e_2)^2 (\Delta f_4 - \Delta f_3)^2 + (e_1 e_3)^2 (\Delta f_4 - \Delta f_2)^2 \\
&+ (e_1 e_4)^2 (\Delta f_2 - \Delta f_3)^2 + (e_2 e_3)^2 (\Delta f_4 - \Delta f_1)^2 \\
&+ (e_2 e_4)^2 (\Delta f_3 - \Delta f_1)^2 + (e_3 e_4)^2 (\Delta f_2 - \Delta f_1)^2]/ \\
&\left[(e_1 e_2 e_3)^2 + (e_1 e_2 e_4)^2 + (e_1 e_3 e_4)^2 + (e_2 e_3 e_4)^2 \right]
\end{aligned}
\tag{1}
$$

where Δf_i is the log ratio of the quantum catches of each class of single cones (long-wavelength sensitive [LWS], medium-wavelength sensitive [MWS], short-wavelength sensitive [SWS], ultraviolet wavelength sensitive [UVS]) denoted by the subscript for cone i, for first ($_1$) and second ($_2$) egg in a contrast.

$$
\Delta f_i = \log \frac{\int_{300}^{700} R_1(\lambda) I(\lambda) S(\lambda) d\lambda}{\int_{300}^{700} R_2(\lambda) I(\lambda) S(\lambda) d\lambda}
\tag{2}
$$

where $R_1(\lambda)$ represents the average reflectance of the target starling egg, $R_2(\lambda)$ is the average reflectance of the contrast elements, either eggs or nest material, in a given clutch, $I(\lambda)$ is the spectral irradiance at the nest, and $S(\lambda)$ is the spectral sensitivity of photoreceptor spectral type i. We relied on published information for single- and double-cone photoreceptor spectral sensitivities, photoreceptor noise, and the transmission properties of avian ocular media for the common starling *Sturnus vulgaris* (Hart et al. 1998; Hart and Vorobyev 2005; see Fig. 1d), which is a close relative of our target signal receiver species. Results of calculations using Eq. (1) provide the chromatic distance ΔS separating the perceptual value of two eggs in starling receptor space.

Evidence suggests that birds may use achromatic signals in discriminatory tasks (reviewed in Kelber et al. 2003), including egg discrimination at low light levels (Avilés 2008). In birds, double cones are assumed to be responsible of achromatic visual detection (e.g., Osorio et al. 1999a, b). Holveck et al. (2010) have recently used the receptor–noise-limited model of chromatic thresholds to describe achromatic discrimination in birds. Here, we followed the same approach and thus applied Eq. 1 to 3 to sensitivity data for double cones for the common starling (Hart et al.

1998; Hart and Vorobyev 2005; see Fig. 1d). We used the Weber fraction as 0.05 (at threshold) for the double cone in birds and calculated achromatic (brightness) contrast distance ΔQ similar to the chromatic analysis. The receptor–noise-limited model, however, was not developed to describe achromatic thresholds, and its use for this purpose has not been tested so far. Birds have in general very high contrast thresholds for achromatic spatial patterns. These thresholds can likely not be explained solely by internal receptor noise in double cones (Kelber et al. 2003). Thus, model outputs concerning achromatic thresholds likely would largely overestimate starling capacity to discriminate based on achromatic cues.

Previous work has shown that detectability of chromatic and achromatic contrasts is highly dependent on model assumptions about photoreceptor noise (Lind and Kelber 2009a; Holveck et al. 2010). Following Holveck et al. (2010), we ran two models with different assumptions about photoreceptor noise. The first model assumed that the signalling noise for each photoreceptor was entirely based on neural noise, thus, for calculations, we assumed that the signalling noise e_i for each cone was independent of light intensity.

$$e_i = \omega/\sqrt{\eta_i} \qquad (3)$$

where ω is the Weber fraction (taken as 0.05 for all single cones (e.g. Théry et al. 2008)) and η_i is the relative density of the cone class i on the retina. We relied on common starling cone proportions of 1:1.38:3.34:3.46 for SWS1/SWS2/MWS/LWS cones, respectively (Hart et al. 1998).

In addition, we ran a second physiological model which considers both neural and quantum photoreceptor noise. This modification of the original model proposed by Vorobyev et al. (1998) was later proposed by Osorio et al. (2004) to deal with the issue that low light conditions may limit visual discrimination due to a high relative importance of neural noise (see also Holveck et al. 2010). As in the first model, we assumed a Weber fraction of 0.05 for all cone types. Depending on the signal received (on the colour seen), the noise is the sum of quantum noise and neural noise (Osorio et al. 2004) and is given by Eq. 4:

$$e_i = \sqrt{\omega^2/\eta_i + 2/(Q_{iA} + Q_{iB})} \qquad (4)$$

where ω is the Weber fraction and η_i is the relative density of the photoreceptor class i on the retina, Q_{iA} is the quantum catch for photoreceptor i and colour A and Q_{iB} is the quantum catch for photoreceptor i and colour B.

The units for ΔS and ΔQ are JNDs (just noticeable differences). As in Cassey et al. (2009), we assumed that values below 1 JND are impossible to discriminate by birds, and those with values below 3 JND would be difficult to distinguish even under favourable light conditions.

We obtained chromatic and achromatic contrasts against the nest background for all the sampled eggs (total of 135 eggs). In addition, we obtained chromatic and achromatic contrasts for all possible pairs of eggs in our sample of 31 nests (i.e. 9,045 combinations of pairs of eggs) and calculated average values of chromatic and achromatic contrasts between pairs of nests by averaging the contrast between all the eggs of the first and second nest in a pair (i.e. 465 combinations of pairs of nests). This allowed us to assess the potential for sexual selection of eggshell colouration based on simultaneous comparisons of coloured clutches in our population. We also explored two different scenarios for perception of egg differences within spotless starling clutches: (1) perception based on maximum values of chromatic and achromatic contrasts among the eggs in a given clutch; and (2) perception based on average chromatic and achromatic differences among all the eggs in a given clutch.

Egg discrimination experiments

The first experiment aimed to interpret differences in egg retrieval in natural nest light conditions by spotless starlings in terms of detectability linked to UV reflectance (Avilés et al. 2006). The experimental approach consisted of the manipulation of UV reflectance with an UV-blocker (see Fig. 1e) of starling eggs introduced outside the nest cup and the study of the retrieval of these eggs (Avilés et al. 2006). Full details on the experimental protocol can be found in Avilés et al. (2006). The second experiment aimed to test whether blue-green colour intensity of artificial model eggs had a significant positive influence on paternal feeding effort (Soler et al. 2008). Briefly, experimental dark and light artificial blue eggs were used to replace original starling eggs and study parental feeding behaviour (see Fig. 1f). Full details on the experimental protocol can be found in Soler et al. (2008). Data from these studies are particularly useful for addressing reliability of visual model predictions in the frame of egg discrimination in holes since they involved manipulation of egg colouration in hole-nest light conditions and report of behavioural responses to these manipulations.

Ethical matters

Experimental eggs used for testing egg retrieval in relation to manipulation of UV reflectance were collected from naturally abandoned starling nests and used fresh after collection (Avilés et al. 2006), therefore causing no changes in eggs' hatchability of tested pairs. Replaced original starling eggs in our second experiment were transferred, matched by phenology, to starling nests that were not used in the experiment (Soler et al. 2008). Once all eggs (i.e.

transferred and original eggs) had hatched in receptor nests, nestlings originated from original eggs were transferred to the experimental nests (Soler et al. 2008). Although nestlings in experimental and receptor nests were never related to the adults taking care of them, they were normally fledged, suggesting a negligible effect of our experiment on the breeding outcome of starlings.

Results

Egg discriminability based on photoreceptor noise-limited performance of single photoreceptors

Visual models where light intensity does not limit detectability showed that eggshell colourations clearly contrasted with nest backgrounds (Fig. 2a, c), suggesting that spotless starlings can see their eggs in the light conditions prevailing at their nests. However, visual model based on the assumption that light intensity limits detectability showed that all calculated contrasts (both ΔS and ΔQ) of the eggs with the nest background fell below the theoretical threshold value for visual discrimination (Fig. 2b, d), suggesting that spotless starlings should not be able to see their eggs in their nests.

From the SSH perspective, the discrimination model where light intensity does not limit detectability indicated that nearly 77% of all possible pairwise clutch comparisons sampled in this study would be perceived as different to the avian visual system of a spotless starling on the base of chromatic discrimination (i.e. $\Delta S > 1$ JND, Fig. 2e). However, a closer examination of contrast values showed that 341 of 465 (73.3%) possible clutch comparisons would be difficult to discriminate (1 JND > ΔS < 3 JND, Fig. 2e). Discrimination based on achromatic signal produced qualitatively similar results since 63.2% of all possible pairs of nests would be perceived as achromatically different at a threshold value of 1 JND (Fig. 2g), although 244 of these 465 (52.5%) clutch comparisons would be difficult to discriminate (1 JND > ΔS < 3 JND, Fig. 2g).

The analyses of chromatic and achromatic contrasts within nests in the model where light intensity does not limit detectability revealed that male spotless starlings also have the potential to discriminate between the eggs of a given clutch. When perception relied on maximum values of contrast, our result showed that spotless starlings could potentially perceive colour differences among the eggs of 20 out of 31 (64.5%) nests (i.e. contrast exceeded the 1 JND threshold criteria; Fig. 2i), although in 19 of these 31 (61.3%) nests discrimination would be difficult (1 JND > ΔS < 3 JND, Fig. 2i). Moreover, in 14 out of 31 (45.2%) nests, maximum achromatic differences exceeded the threshold value of 1 JND for

discrimination, although in all cases achromatic differences were below the threshold value of 3 JND (Fig. 2k). When perception relied on average differences within the clutch, we found that in seven (22.6%) and three (9.7%), respectively, out of 31 nests, the average chromatic and achromatic contrasts exceeded the 1 JND threshold for discrimination (Fig. 2m, o). However, chromatic and achromatic contrast never exceeded the threshold value of 3 JND suggesting that at best average differences in colouration of spotless starling eggs within a clutch would be difficult to discriminate.

Visual model based on the assumption that light intensity limits detectability showed that all pair-nest comparisons predominantly fell below the theoretical threshold value for visual discrimination (Fig. 2f, h, j, l, n, p), suggesting that spotless starlings should not be able to distinguish between eggs of different clutches.

Behavioural tests of predictions on egg discriminability based on photoreceptor noise-limited performance of single photoreceptors

Discrimination ability based on visual models where light intensity does not limit detectability revealed that chromatic and achromatic contrasts between tested stimuli in behavioural discrimination experiments (Avilés et al. 2006; Soler et al. 2008) in spotless starlings fall largely above the threshold values for discrimination (Table 1), therefore, leading to the prediction of high discriminability. By contrast, the model based on the assumption that light intensity limits detectability generated the opposite prediction since chromatic and achromatic contrasts fall largely below the threshold values for discrimination (Table 1), suggesting null discrimination.

Behavioural experiments fitted the prediction of the visual model where light intensity did not limit detectability since manipulations of signal intensity emitted by eggs at the ultraviolet and blue wavebands (Fig. 1 e, f) provoked significant behavioural discrimination (Table 1).

Discussion

Our results suggest that visual modelling cannot clarify whether starlings can detect their eggs in the context of the SSH because egg detectability by starlings depended entirely on model assumptions of visual limitations under dim light conditions. Indeed, when we relied on a physiological model that specifically deals with the issue that low light conditions may limit visual discrimination due to a high relative importance of neural noise (Osorio et al. 2004), calculated colour and brightness contrasts between eggs of different clutches and between eggs and

Fig. 2 Variation in eggshell colouration quantified by realistic models of retinal function across and within spotless starling clutches. Frequency of eggs in relation to chromatic and achromatic contrasts with the nest background with photoreceptor noise either **a**, **c** independent or **b**, **d** dependent of light intensity. Frequency of pair of nests in relation to chromatic and achromatic comparisons of clutches with photoreceptor noise either **e**, **g** independent or **f**, **h** dependent of light intensity. Number of nests in relation to maximum differences within the clutch in chromatic and achromatic contrasts with photoreceptor noise either **i**, **k** independent or **j**, **l** dependent of light intensity. Number of nests in relation to average differences within the clutch in chromatic and achromatic contrasts with photoreceptor noise either **m**, **o** independent or **n**, **p** dependent of light intensity. *White bars* are for contrasts above 3 JNDs, *grey bars* for contrasts below 3 JNDs and above 1 JND, *black bars* for contrasts below 1 JND
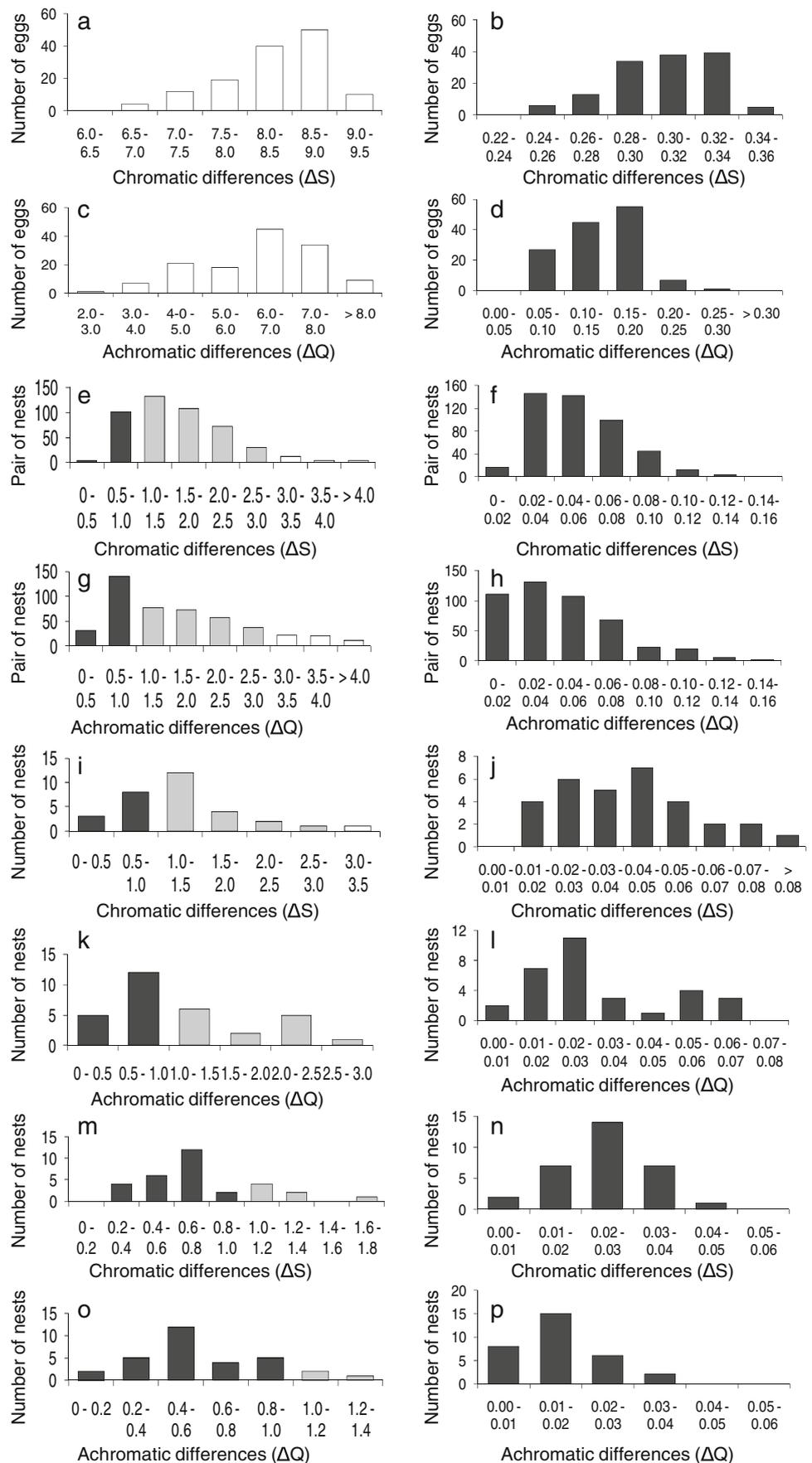
Table 1 Behavioural tests of predictions on egg colour discrimination arisen from a photoreceptor noise-limited colour opponent model of avian perception for spotless starlings

Study	Stimulus	Photoreceptor noise is independent of light intensity		Model prediction	Photoreceptor noise is dependent of light intensity		Model prediction	Behavioural response measured within the nests
		Chromatic contrast (JND)	Achromatic contrast (JND)		Chromatic contrast (JND)	Achromatic contrast (JND)		
Avilés et al. (2006)	UV-blocked versus horse fat-coated starling eggs	39.11	0.13	High detectability	0.425	0.001	Null discrimination	Ultraviolet-reflecting eggs were more retrieved than UV-blocked eggs
Soler et al. (2008)	Dark versus pale experimental eggs	13.96	11.50	High detectability	0.20	0.08	Null discrimination	Males from nests with experimental dark eggs fed the nestlings more frequently than those from nests with experimental pale eggs

Chromatic and achromatic contrasts for tested stimulus were calculated with two visual models assuming that photoreceptor noise was either independent (i.e. light intensity did not limit detectability) or dependent (i.e. light intensity may limit visual performance in dim light conditions) of light intensity (Osorio et al. 2004; see Methods for further details) based on average spectrophotometric data and irradiance values reported in Fig. 1. Model predictions on egg discriminability dwells on empirical studies performed in lab conditions showing that chromatic and achromatic contrasts with values <1 JND (just noticeable difference are impossible to discriminate; Vorobyev et al. 1998; Goldsmith and Butler 2005)

the nest material fell below the predicted thresholds for discrimination, suggesting that spotless starling would not be capable of detecting differences in colouration between eggs of different cavity-hole nests. However, when model assumptions about detectability linked to light intensity were relaxed (i.e. light intensity did not limit detectability), contrasts emerging from the visual model largely fell above the threshold value for discrimination suggesting that starlings could potentially perceive as different many of possible pairwise clutch comparisons. A previous study using a similar visual model approach that accounted for visual performance limitation in low light conditions yielded qualitatively identical results in a test of egg detectability in great tits (Holveck et al. 2010).

Here, for the first time, we have tested the predictions on eggshell detectability that emerged from visual models under dim light by analysing spectrophotometric data collected in two egg-discrimination experiments in spotless starlings. Interestingly, model predictions emerging under the assumption that light intensity limited visual performance were not fulfilled by behavioural experiments since manipulations of signal intensity emitted by eggs at the ultraviolet and blue wavebands, which were predicted largely undetectable by the visual model, provoked significant behavioural discrimination in starlings (Table 1). Results of behavioural experiments, however, fitted the prediction of the visual model where light intensity did not limit detectability. Previous studies have already reported inconsistencies between visual model predictions and behavioural tests of real discrimination under low light conditions (Vorobyev et al. 1998; Lind and Kelber 2009b). However, the inconsistencies between model predictions and behavioural experiments reported here are most probably due to the use of chromatic models to predict behavioural results measured in dim light. In dim light, achromatic mechanisms may become more important (Kelber et al. 2003). This leaves us with the possibility that starlings possessed an efficient visual system endowed with compensative mechanisms of visual discrimination based on achromatic cues operating under low light conditions which may lead to the mismatch between modelling and empirical results.

Under low light conditions, there could be several physiological mechanisms that could efficiently compensate for restricted light and enhance visual performance, and that are not accounted for in the colour opponency model of Vorobyev and Osorio (1998). For instance, photon capture may be improved neurally by summing the outputs of neighbouring visual channels (spatial summation) or by increasing the length of time a sample of photons is counted by the eye (temporal summation) (Warrant 1999). Also, recent studies suggest that sensitivity measurements should be expanded to also consider receptor transduction mech-

anisms and post-receptor processing in order to provide reliable predictions of avian visual thresholds in the mesopic range (Lind and Kelber 2009b). In addition, it is possible that the rods, which encode achromatic and not chromatic information, and which are present in the retina of starlings (Hart 2001)) could partly compensate the contribution of cones (limited by dim light conditions; Reitner et al. 1991; Vorobyev and Osorio 1998). Avilés et al. (2006) explained that birds detecting eggs outside the nest cup were given 2 h to retrieve the eggs. This will give significant time for adaptation and a contribution from the scotopic rods (see Reynolds et al. 2009). Unfortunately, visual models that incorporate the contribution of rods have not been developed yet, which precluded testing this possibility.

Altogether, previous experimental work and the present visual model calculations of discrimination ability suggest that the sole use of noise-limited detection thresholds of single photoreceptors to predict the intensity threshold of egg colour discrimination in the context of the SSH is inadequate (see also Holveck et al. 2010). Given that birds still can discriminate between visual stimuli that, according to the use of photoreceptor noise-limited models of visual performance, would not be discriminable (this study), future studies aiming to establish thresholds values for visual colour discrimination should employ behavioural discrimination tests if at all possible.

In conclusion, although the physiological mechanisms behind egg discriminability in cavity nests merit further investigation, low light conditions in cavity nests do not constrain detectability of egg colour differences in spotless starlings (Table 1). These findings add to previous reported evidence showing that feeding parents can use subtle differences in nestling colouration as feeding cues in cavity nests (Heeb et al. 2003; Jourdie et al. 2004; Bize et al. 2006; Kilner 2006). Visual models of colour discrimination dependent on light intensity suggested that starlings cannot see their eggs in their nests (this study, see also Holveck et al. 2010), suggesting that there must be other physiological mechanisms at work different from colour discrimination based on single photoreceptors. The model for dim light used in this paper providing contrast value ΔQ only considers contribution of the photopic double cones. Future studies testing constraints for the use of egg colouration in the context of the SSH should combine both modelling and behavioural experiments to determine what are the components of the models (i.e. achromatic and/or chromatic) that produce the mismatch with behavioural conditions by manipulating reflectance of the eggs and testing these manipulations behaviourally.

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