Can eggs in a cavity be a female secondary sexual signal? Male nest visits and modelling of egg visual discrimination in blue tits

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Eggshell coloration is thought to function as a female-specific secondary sexual trait. While tests of this idea are rapidly accumulating in cavity-nesting birds, some fundamental underlying assumptions remain rarely investigated: namely, can males see eggshell coloration and perceive colour differences between the eggs of different females? We tested these two key assumptions in a natural population of blue tits (*Cyanistes caeruleus*). Using transponders, we tracked male nest visits and found that all males visited their nest-boxes while eggs were present and often visually accessible. Interestingly, some males also visited neighbouring nests. We then tested whether birds could detect eggshell coloration using models of avian colour vision; models were performed with and without limitations on visual performance owing to dim light. Both models found that differences in eggshell brightness were often easier to discriminate than differences in colour; there was more contrast in white eggshell background between clutches than within and its contrast against nest background was repeatable within clutches, suggesting these features could act as signals. Yet, the detectability of these contrasts depended entirely on model assumptions of visual limitations. Consequently, we need a better understanding of underlying visual mechanisms in dim-light environments and behavioural discrimination experiments before confirming the signalling potential of eggshell coloration.

Keywords: eggshell coloration; sexual signal; nest visit; visual perception; *Cyanistes caeruleus*

1. INTRODUCTION

Bird eggshell colour diversity has always puzzled evolutionary biologists. Until recently, it was believed to be evolutionarily driven mainly by predation (Kilner 2006). However, sexual selection may also be

implicated since eggshell coloration could signal a female's quality and/or investment and affect paternal investment in the offspring (Moreno & Osorno 2003). Few studies have hitherto investigated this hypothesis and their findings have been mixed (Reynolds *et al.* 2009). This result may arise in part because most studies have overlooked whether males (i) have the opportunity to see their (and conspecifics') clutches and (ii) perceive colour differences between the eggs of different females. Such assumptions should be validated before claiming that sexual selection is operating.

The issue of visual perception is of particular concern given that most studies have focused on cavity-nesting species, for which dim-light intensity in the nest may limit detectability. Only two studies have assessed eggshell coloration from the male perspective, and they concluded that the potential for sexual signalling was limited (Cassey *et al.* 2009) or unlikely (Cassey *et al.* 2008). Moreover, evidence that males have visual contact with their eggs (or those of others) is scant for solitary breeders nesting in cavities (Reynolds *et al.* 2009; but see Moreno *et al.* 2005).

First, we investigated whether males have the opportunity to see their eggs by using transponders to monitor their daily nest visits and by checking whether or not eggs were visually accessible. We then used visual models to examine whether males can discriminate differences in eggshell colour and brightness between clutches given the dim-light conditions in the nest. Our biological model was the blue tit *Cyanistes caeruleus*. In this cavity-nesting passerine with biparental care, eggshell pigmentation patterning and colour correlate with female state (Martínez-de la Puente *et al.* 2007; Sanz & Garcia-Navas 2009) and with paternal effort to feed offspring (Sanz & Garcia-Navas 2009).

2. MATERIAL AND METHODS

We conducted the study in an artificial nest-box plot (43°40' N, 03°40' E) in southern France (Blondel *et al.* 2006). We equipped birds with transponders to monitor their daily activity at six nest-boxes in 2008 and 44 in 2009. Nest visits were recorded from the end of the nest-building phase to the completion of incubation (electronic supplementary material). We tested whether males' visits differed between the laying and incubation phases using a linear mixed model with year (nest(male)) as a nested random factor. We also investigated whether eggs were visually accessible or covered by nesting material and/or females (electronic supplementary material).

Using spectrometry (over 300–700 nm), we characterized the white eggshell background of 479 eggs from 42 clutches and the brown eggshell spots of 31 eggs from 30 clutches collected in 2007 (electronic supplementary material). Using the protocol of Loyau *et al.* (2007), we computed six types of colour (ΔS) and brightness (ΔQ) contrasts (Vorobyev & Osorio 1998) to assess whether males (i) can see eggshell colour and brightness in a cavity and (ii) can discriminate between different eggs within and between clutches. We assessed (i) egg detectability in nest-boxes by contrasting the white eggshell background against the nest background (a) (electronic supplementary material, figure S1), the brown spots against the nest background (b) and against the white eggshell (c) and (ii) between-egg detectability by contrasting the white eggshell of paired eggs between (d) and within clutches (e) and the brown spots of paired eggs between clutches (f).

Contrasts expressed in just-noticeable differences (JNDs) were compared with the discrimination threshold, below which colour (or brightness) differences are undistinguishable and above which they gradually become more distinguishable for larger contrast values. We modelled both a highly and a poorly performing visual system by setting the threshold at 1 and 2 JNDs, respectively (Eaton 2005). We also assumed that light intensity did (neural and quantum noise ΔS_d , ΔQ_d) or did not (neural noise ΔS_{ind} , ΔQ_{ind})

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Figure 1. Male blue tits' opportunities to see eggs in 2008–2009. (a) Daily patterns of males' within- and extra-pair nest visits. Shown are means (solid line) $\pm$ 1 s.e.m. (dotted line). Each arrow shows the extra-pair visits (in absolute frequency per day) of one neighbouring male ($n = 11$). (b) Percentages of nests with an egg (or several eggs) either uncovered (i.e. visible; in white), slightly covered (i.e. still visible; pale grey) or totally covered (i.e. invisible; dark grey) over the course of the laying sequence. De0, day of the first laid egg; Di0, day of clutch completion in all monitored nests. n.a., not applicable.

limit visual performance; the latter might occur if physiological mechanisms (e.g. photoreceptor response pooling, contribution of rods and cones) compensate for low photon capture in nest dim-light conditions (electronic supplementary material).

We tested whether contrasts differed from the discrimination threshold with one-sample *t*-tests (or Wilcoxon signed-rank tests when distributions were not normal). Within each comparison (a–f), ΔS was compared with ΔQ using linear mixed models with nest(egg) as a nested random factor (a–c) or contrast type as a random factor (d–f). Within each type of Δ (*S* or *Q*), we compared the contrasts (d, e) using linear models and the contrasts (a–c) using linear mixed models with nest(egg) as a nested random factor. We ran post hoc tests for multiple comparisons of means (Tukey contrasts) and report adjusted *p*-values. The two model types (Δ_{ind} and Δ_{d}) were analysed separately. We compared between- to within-clutch variation of white eggshell background contrasted against nest background with repeatability estimates $R \pm 1$ s.e. (electronic supplementary material). Data were analysed in R software v. 2.9.2 (R Development Core Team 2009). Models were two-tailed with $\alpha = 0.05$.

3. RESULTS

All males visited their nest over the laying and incubation phases. Male parental visit rates were

consistent within both laying and incubation phases, but increased sharply between the two phases (estimate $\pm$ s.e. = 0.23 ± 0.02 , $F_{1,297} = 87$, $p < 0.001$), starting at the end of the laying phase (figure 1a; electronic supplementary material, table S1). We recorded 61 extra-pair nest visits (figure 1a) from 11 neighbouring males (two in 2008, nine in 2009).

Eggs were uncovered in about 50 per cent of the nest-boxes over the laying phase in 2009 and in more than 50 per cent of boxes in the first and last laying days (figure 1b). During incubation in 1998–1999, 25 per cent of the birds found on eggs were males (1998: one instance out of four nests; 1999: two out of eight).

In both visual models (Δ_{ind} and Δ_{d}), brightness contrasts ΔQ were larger than colour contrasts ΔS (a, b, d, e) (all $p < 0.001$; figure 2a–d), except for brown spots contrasted against white eggshell (c) ($\Delta Q < \Delta S$: both $F_{1,30} > 67$, $p < 0.001$) and brown spots contrasted between clutches (f) ($\Delta Q_{\text{ind}} = \Delta S_{\text{ind}}$: $F_{1,434} = 0.18$,

Figure 2. (a,c) Colour and (b,d) brightness contrasts of white eggshell background against nest background (shell/nest), brown eggshell spots against nest background (spot/nest), brown spots against white eggshell (spot/shell), white eggshell between clutches (shell/shell_{BC}), within clutches (shell/shell_{WC}) and brown spots between clutches (spot/spot_{BC}) with photoreceptor noise either (a,b) independent or (c,d) dependent of light intensity. Shown are means $\pm$ 1 s.d. The dotted lines show the discrimination threshold of 1 JND. White bars are for contrasts significantly above 2 JNDs, grey bars for contrasts significantly below 1 JND. *** $p < 0.001$.

$p = 0.7$; $\Delta Q_d < \Delta S_d$: $F_{1,434} = 78$, $p < 0.001$). Egg detectability in nests (a–c) differed between brightness or colour contrast types (all $F_{2,59,438} > 341$, $p < 0.001$; figure 2a–d), suggesting some contrasts are easier to detect than others.

In both visual models, we found significant between-clutch differences in eggshell colour and brightness: the contrasts of white eggshell background between clutches (d) were larger than within clutches (e) (all $F_{1,6004} > 132$, $p < 0.001$; figure 2a–d). Furthermore, the contrasts of white eggshell background against nest background (a) were repeatable within clutches (all $F_{41,437} > 8.9$, $p < 0.001$,

$R \pm \text{s.e.} > 0.4 \pm 0.06$), implying a possible signalling function of eggshell coloration.

In visual models where light intensity did not limit detectability (Δ_{ind}), most contrasts exceeded the conservative discrimination threshold of 2 JNDs with $p < 0.001$ (figure 2a,b), suggesting high detectability. When light intensity was limiting (Δ_d), the results remained qualitatively similar, but all contrasts fell below 1 JND (all $p < 0.001$; figure 2c,d), suggesting undetectability. Only the brightness contrast ΔQ_d of white eggshell background against nest background (a) could be distinguished for some eggs (43% of the eggs

exceeded 1 JND; electronic supplementary material, figure S2).

4. DISCUSSION

All monitored males visited their nest multiple times when eggs were present and eggs were often uncovered. Moreover, a large proportion of males visited other nests. They thus had ample opportunities to view and potentially assess eggs, which validates the first assumption underlying the signalling potential of eggshell coloration.

Male within-pair nest activity increased and became more variable between males as the laying phase ended. This observation suggests the very interesting possibility that males modulate their reproductive investment as early as in the incubation phase (e.g. in varying the courtship feeding of their incubating female partner; Nilsson & Smith 1988; but see Sanz & Garcia-Navas 2009).

Studies to date suggest that brown pigmentation may be used by males to adjust their parental behaviour (Martínez-de la Puente *et al.* 2007; Sanz & Garcia-Navas 2009). We suggest white eggshell coloration is also a good candidate: it contrasted more between than within clutches; its contrast against the nest background was repeatable within clutches; and it remained informative even when light intensity limited visual system performance (electronic supplementary material, figure S2). High between-clutch variability in eggshell coloration supports a signalling role for eggshell coloration. However, visual modelling does not clarify whether blue tits can detect these differences as detectability depended entirely on model assumptions of visual limitations under dim-light conditions.

In order to validate the assumption that eggshell coloration differences are detectable and thus could act as a sexual signal, physiological processes that compensate for low photon availability must be identified. Some mechanisms, like the pooling of photoreceptor responses (Warrant 1999) or the contribution of rods to cone responses (Reitner *et al.* 1991; Vorobyev & Osorio 1998), are known to operate when light is dim, but a lack of physiological data in birds currently prevents progress in visual modelling. Nevertheless, the hypothesis that birds maintain visual acuity in the nest cavity is supported by findings that bird behaviour changes if egg or chick coloration is manipulated (e.g. Jourdie *et al.* 2004; Soler *et al.* 2008). Moreover, birds might exploit brightness, since it seems easier to detect, than colour in dim-light environments (this study; Gomez & Théry 2007; Avilés 2008) and is implicated in many visual tasks (Kelber *et al.* 2003). Our results underscore the need for further physiological, behavioural and modelling exploration of visual performance in the real conditions of the nest.

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