

SHORT COMMUNICATION

The intensity threshold of colour vision in a passerine bird, the blue tit (*Cyanistes caeruleus*)

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ABSTRACT

Many vertebrates use colour vision for vital behaviour but their visual performance in dim light is largely unknown. The light intensity threshold of colour vision is known only for humans, horses and two parrot species. Here, we first explore this threshold in a passerine bird, the blue tit (*Cyanistes caeruleus*). Using classic conditioning of colour cues to food rewards in three individuals, we find a threshold ranging from 0.05 to 0.2 cd m⁻². Results are comparable to the two previously tested bird species. For tits, nest light conditions probably exceed that threshold, at least after sunrise. These results shed new light on the lively debate questioning the visual performance of cavity nesters and the evolutionary significance of egg and chick coloration. Although this needs further investigation, it is possible that blue tits exploit both colour and brightness cues when viewing their eggs, chicks or conspecifics in their nests.

KEY WORDS: Colour vision, Cavity-nesting bird, Conditioning experiment, Egg coloration, Bird coloration, Communication

INTRODUCTION

Light intensity varies widely from dim starlight to bright sunny days. In such a range of conditions, colour vision – discrimination of spectral composition irrespective of brightness – is more stable than achromatic vision, because of colour constancy. In dim light, colour vision is limited by photon capture and signal-to-noise ratio and is often sacrificed for enhanced brightness sensitivity (reviewed in Kelber and Roth, 2006). Most vertebrates use cones for colour vision in bright light, rods for brightness detection in very dim light and both at mid light levels (mesopic range). The lower intensity limit of cone functioning sets the intensity threshold of colour vision, and is known in four species: humans (~0.02 cd m⁻²) (Wyszecki and Stiles, 1982), horses (0.02–0.08 cd m⁻²) (Roth et al., 2008), two parrot species (0.1–0.4 cd m⁻²) (Lind and Kelber, 2009), which fall between levels equivalent to moonlight (0.01–0.1 cd m⁻²) and twilight (0.3–10 cd m⁻²).

Many diurnal vertebrates rely on vision for vital behaviour and can be active in dim light environments (cavities, dense forest cover, dawn, dusk). Nevertheless, little is known about their vision in such light conditions, which is crucial knowledge for understanding animal communication. For example, there is a debate concerning the intensity limit of the loss of colour vision in birds (Holveck et al., 2010; Avilés et al., 2011). Many experiments in cavity-nesting birds (mostly passerines) show that parents adjust their behaviour to

colour-manipulated eggs (Moreno et al., 2006; Martínez-de la Puente et al., 2007; Sanz and García-Navas, 2009; Antonov et al., 2011; Avilés et al., 2011) or chicks (Jourdie et al., 2004; Bize et al., 2006; Tanner and Richner, 2008; Dugas, 2009; Wiebe and Slagsvold, 2009). Whether they use colour and/or achromatic cues remains unknown (but see Antonov et al., 2011). Some authors argue that nest light conditions make colours unexploitable (Wesołowski and Maziarz, 2012). Visual models (Vorobyev and Osorio, 1998) – used for dim light but unvalidated for such light conditions – predict no colour discriminability (Holveck et al., 2010; Avilés et al., 2011). However, nest light conditions may fall in birds' mesopic range. Which photoreceptors may contribute to colour vision in mesopic conditions remains poorly understood (Kelber and Roth, 2006).

Although passerines represent ~60% of avian species and regularly experience dim light conditions (e.g. Thomas et al., 2002), their colour vision threshold remains unknown. Here, we determine the intensity threshold of colour vision in the blue tit *Cyanistes caeruleus* Linnaeus 1758. This cavity-nesting passerine frequently faces dim light conditions (Amrhein et al., 2008; Steinmeyer et al., 2010). It is known for its vision (Hart et al., 2000) and is often considered as a representative species with high ultraviolet sensitivity in visual modelling (Antonov et al., 2011). Our study will help to identify which visual cues are available for parents to exploit, which would thus be potential targets of selection.

RESULTS AND DISCUSSION

We found an intensity threshold of colour vision ranging from 0.05 cd m⁻² to 0.2 cd m⁻² in three tested individuals (Fig. 1). Similar values of around 0.4 cd m⁻² and 0.1 cd m⁻² have been obtained in Bourke's parrots and budgerigars (Lind and Kelber, 2009), with comparable sample sizes. Our results are interesting in the current debate concerning vision performance of cavity-nesting birds and the evolution of egg or chick coloration (Reynolds et al., 2009). For egg coloration, although crypsis and brood parasitism have long been considered to drive eggshell coloration, the recent hypothesis suggesting that it could evolve through sexual selection as a signal to males of female quality is considered unlikely for cavity-nesting birds, given light limitations and the lack of robust experimental evidence (Cherry and Gosler, 2010). Light levels within natural nests of marsh tits and great tits (with similar nesting habits to blue tits) are recorded as 1–10% of the light levels outside (Wesołowski and Maziarz, 2012). For blue tits to detect colours in their nest, light levels outside their nest should be between 0.5 and 20 cd m⁻². Civil twilight corresponds to 1–10 cd m⁻² (Kelber and Roth, 2006). Hence, it would be possible for blue tits (even our worst-performing individual) to exploit colour and brightness cues at their nest, during daytime. Blue tits can be observed around their nest 1 h before sunrise (A.G., R. Guerreiro and M.-J. Holveck, unpublished data) and they are active throughout the day, visiting their own nest and

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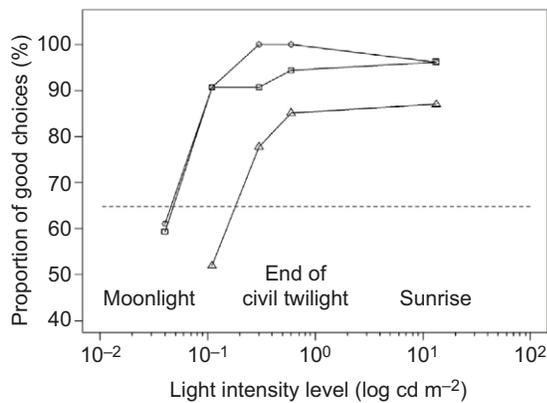


Fig. 1. Proportion of good choices made by the blue tit (*Cyanistes caeruleus*) in relation to light intensity. Values are expressed as $\log \text{cd m}^{-2}$ from sunrise to moonlight. We tested three different individuals (Female 1, triangle; Female 2, circle; male 1, square) at decreasing light intensities. The dashed line represents the line obtained for $P=0.05$ obtained in a two-tailed binomial test. Below the $P=0.05$ line, bird choices are not statistically different from random choices, determining the intensity threshold of colour vision (two-tailed binomial test).

the nests of their neighbours when eggs are present (Holveck et al., 2010). If they do use colour vision, they could select for colour cues in eggs and chicks.

Further studies should question two crucial points: dark adaptation and the use of colour vision in natural conditions. First, blue tits visit their nest for 10–90 seconds when feeding their nestlings (A.G., R.G. and M.-J.H., unpublished data), hence undergoing rapid changes in light intensities. Even if capable of colour vision, blue tits could exploit colour cues only if they are able to perform rapid dark adaptation. No data are available on whether they can do this. Second, differences in coloration between eggs or chicks, or between nests (or even within nests) are subtler than differences between the cards used in our experiment. The subtler the differences, the more difficult the information can be exploited in dim light. Even if colour vision can be used at nest light levels, it remains unknown whether refined colour differences can be discriminated and used to adjust behaviour. Experimental studies altering egg or chick coloration in cavities in tits and other species, showed a change in parental behaviour manipulated reflectance over the UV range (Jourdie et al., 2004; Bize et al., 2006; Dugas, 2009; Avilés et al., 2011) or over a broad wavelength range (Wiebe and Slagsvold, 2009; Antonov et al., 2011). However, these studies did not determine which cues were used, except in one case, where birds probably used brightness (Antonov et al., 2011). Manipulation of coloration irrespective of brightness, within natural ranges of variation, is indispensable to determine whether birds use colours in dim conditions.

The blue tit is likely to have a good visual performance. Blue tits have multifocal optics, which correct for chromatic aberration and improve colour vision more efficiently than monofocal optics (Lind et al., 2008). Relating eye size to body mass and time of singing onset, Thomas et al. (Thomas et al., 2002) find that passerines with larger than expected eye size start singing earlier and have a greater visual performance. Using their data and results, we found that cavity nesters such as *Cyanistes caeruleus*, *Parus major*, *Parus palustris*, *Passer domesticus*, *Sturnus vulgaris* or *Ficedula hypoleuca* have an eye size 1.3- to 2-times larger than expected (1.8–2 for *Cyanistes caeruleus*), which suggests they have a greater visual performance than average [57 passerines tested (Thomas et

al., 2002)]. Whether this visual performance is specific to cavity nesters deserves further investigation.

In summary, our study strongly suggests that it is possible for blue tits to exploit colour and brightness cues, at least during the daytime. It adds important information to the current debate of bird vision and opens new research paths to experimentally test whether colour is involved in intraspecific communication in dim light conditions.

MATERIALS AND METHODS

Animals

We tested colour vision in three blue tits: two adult females (>1 year old) and one 6-month-old male, a sample size similar to previous studies investigating light threshold for colour vision [three in the horse (Roth et al., 2008); three in the Bourke's parrot and six in the budgerigar (Lind and Kelber, 2009)]. The wild-caught birds were kept in outdoor aviaries with food *ad libitum* (mealworms, fresh apples, seeds and cake), in conditions complying with French regulations (research program permit 201267-003; CEFÉ permit B34-172-11).

Testing room

We placed birds individually in a light-safe testing room, with black-painted walls ($H \times L \times W$: $2 \times 2 \times 1$ m), and controlled diffuse ceiling lighting (30 regularly spaced white LEDs, Nichia NSPL515DS) timed on a 14h:10h light:dark photoperiod to ensure they had enough time to eat. We placed two deep feeders ($18 \times 12 \times 7$ cm) in a wall, 1 m below the ceiling, and monitored bird movements with an infrared camera (Sony HDR-SR7E) in the opposite wall. The light level was controlled by using a variable resistor. We performed luminance measurements using a white paper placed horizontally (if placed vertically, measurements yielded similar values) just below the feeders, and a luminance meter (LMT 1009). We explored five different light intensity levels, ranging from 13.25 to 0.04 cd m^{-2} (13.25, 0.60, 0.29, 0.11, 0.04), equivalent to late day to moonlight, which are ecologically relevant dim light conditions experienced by wild blue tits (Fig. 2).

At all times, the bird could access a water supply, a nest box and various perches, and mealworms placed in the feeders, deep enough so that the bird could not view them from outside. During conditioning and testing, the bird had access only to mealworms in feeders to increase its motivation to feed from the feeders. Otherwise – which represented at least 2 h per day – it had access to apple, muesli, mealworms and seeds on the cage floor.

As soon as a bird started to feed routinely from feeders, we started training it to discriminate blue from green, green being the rewarding colour, following the same protocol and learning criteria as Lind and Kelber used for parrots (Lind and Kelber, 2009). Both feeders contained mealworms and were associated with a colour card: green card associated with accessible food, blue card associated with food made inaccessible by the presence of a metallic grid just above the mealworms, which were thus not viewable from outside. After a choice, we changed the position of the cards and the grid associated with the blue card from outside the room, with minimal disturbance to the bird.

Conditioning and testing protocol

We followed the conditioning protocol established by Lind and Kelber (Lind and Kelber, 2009). We first trained the birds at the highest luminance level by using sets of 18 card pairs. We considered a bird was efficiently trained and used colours to choose the feeder, when it made at least 14/18 correct choices in two consecutive sets of 18 card pairs (77% correct choices, corresponding to $P=0.05$ in a two-tailed binomial test). We then proceeded to testing, at decreasing luminance levels, starting from the highest level. After at least 30 minutes of acclimatization, we presented the bird with three consecutive sets of 18 presentations (54 tests). When the bird made more than 64.8% correct choices (35 correct choices out of 54, $P<0.05$ in a two-tailed binomial test), we considered it was still able to detect colours and we tested it at the next lowest light level. Otherwise, if the two-tailed binomial test showed that choice was not different from a random choice, we considered that the bird did not use colour vision at the tested light level, and we did not test the bird at the next lowest light level. The bird was returned to its aviary before release. Female 1 entered the feeders

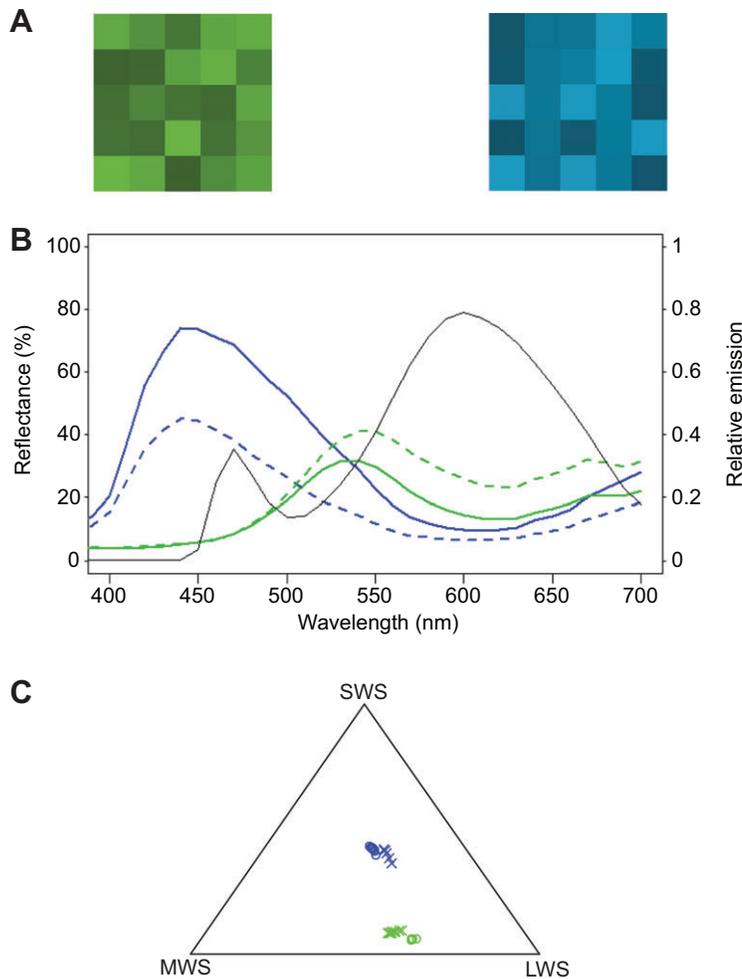


Fig. 2. Card pairs used for vision condition and testing.

(A) Example of blue and green cards of a pair (dc4, see Table 1). (B) Example of reflectance spectra for two card pairs matched for brightness detection, dc4 matched for double cones (plain blue and green lines) and r3 matched for rods (dashed blue and green lines). Relative emission spectrum of the white LEDs used in the experiment (plain black line). (C) Chromaticity diagram representing the receptor space of the relative quantum catches (a quantum catch is normalised to the sum of all quantum catches) for short-wavelength-sensitive (SWS), middle-wavelength-sensitive (MWS), and long-wavelength-sensitive (LWS) receptors. Quantum catches are computed as in Vorobyev and Osorio (Vorobyev and Osorio, 1998); hence they are not corrected for any adaptive background. The ultraviolet-sensitive cone was ignored on the plot (but not in calculations) because its response was very low relative to that of the other receptors. Blue and green pairs matched for double cones (cross symbols) and rods (circle symbols) are shown.

immediately after being put in the test room but took 309 tests before showing effective learning. By contrast, Female 2 and Male 1 took 22 and 10 days, respectively, to enter the feeders but immediately showed effective learning.

Visual stimuli

We built 13 pairs of blue and green cards. Each trial consisted in presenting a blue and a green card from the same pair (see Fig. 2A). Each set of 18 card presentations was organised in two consecutive series of nine card

Table 1. Visual stimuli built for vision testing in the blue tit (*Cyanistes caeruleus*)

	Blue card			Green card			ΔS
	B-value in RGB	G-value in RGB	QC	G-value in RGB	R-value in RGB	QC	
Double cones							
dc1	109	87	181	109	65	198	46.06
dc2	130	104	214	119	71	213	48.23
dc3	140	112	230	129	77	229	49.66
dc4	150	120	246	139	83	245	50.97
dc5	160	128	262	149	89	261	52.08
dc6	170	136	278	160	96	278	52.71
dc7	180	144	294	170	102	294	53.23
dc8	190	152	310	180	108	309	53.71
Rods							
r1	80	48	102	117	93.6	102	55.11
r2	90	54	118	133	106.4	117	58.93
r3	100	60	134	150	120	133	61.5
r4	110	66	149	166	132.8	149	63.69
r5	120	72	165	182	145.6	164	65.58

We built eight pairs for double cones and five pairs for rods. For each pair, RGB values for the channels that had non-null values are shown. The R-channel was 0 for the blue colours, the B-channel was 0 for the green colours. The RGB values followed the proportions given in the Materials and methods. For example, for dc1, we used the proportions: red=0, green=0.8 and blue=1 for the blue card; RGB values were therefore 0, 87 and 109, respectively. We also present the quantum catch values (QC) in arbitrary units for the system responsible for brightness detection. Finally, the colour contrast (ΔS) between the cards of a pair, computed as in Vorobyev and Osorio's model (Vorobyev and Osorio, 1998) in its log formulation (Osorio et al., 2004) was computed, using neural noise as the noise in the system.

presentations. Cards consisted of chequered patterns (5×5 cm) of 25 squares of different brightness values around an average value. We explored a large range of brightness values for these averages (Table 1). We used chequered patterns to avoid birds using brightness cues to discriminate feeders. For each pair, we computed the average values so that the green and the blue card would yield the same brightness impression for a blue tit, i.e. the same quantum catch for the photoreceptor responsible for brightness detection (Table 1). In the blue tit, brightness is detected by double cones in bright light, and rods in nocturnal conditions (Hart et al., 2000). Because we targeted the mesopic range where cones and/or rods may contribute to vision, we had to adjust brightness for both types of photoreceptors. We adjusted eight pairs of cards for double cones, and five for rods. As the light level decreased, we tested an increasing number of cards adjusted for rods (from 1 to 5 for levels from 13.25 to 0.04).

We computed quantum catches Q as:

$$Q = \int I(\lambda)R(\lambda)S(\lambda)\delta\lambda, \quad (1)$$

where I is the irradiance spectrum of the white LEDs, R is the reflectance of a colour patch and S is the absorbance spectrum of the photoreceptor responsible for brightness detection. We first generated a set of colours, each obtained by a given RGB proportion. We measured their reflectance using an Avantes spectrometer and a deuterium halogen light source. We computed rod and double cone quantum catches using physiological data on spectrum absorbance, oil droplet filtering, ocular media transmission (Hart et al., 2000; Hart and Vorobyev, 2005) and templates for photoreceptors and oil droplet filtering (Govardovskii et al., 2000; Hart and Vorobyev, 2005). We established the regression line for each colour, and retained the blue and green colours that were closest in slope to create the maximal number of possible pairs.

For cards adjusted for double cone vision, we used the proportions (red=0, green=0.8 and blue=1) for blue and (red=0.6, green=1, blue=0) for green. For cards adjusted for rod vision, we used the proportions (red=0, green=0.6 and blue=1) for blue and (red=0.8, green=1, blue=0) for green. We used the average brightness values to generate the chequered patterns by using a purpose-built program. Each card presented an internal contrast of 0.3 and 25 squares of different brightness values around the average computed. As patterns were generated at random for each card, all cards within a pair differed in their patterns. Data used for cards are presented in Table 1, with an example of the reflectance spectra in Fig. 2B and the location of all pairs in the chromatic space in Fig. 2C.

For each set of 18 card pair presentations (organised in two consecutive series of nine card pairs), we randomly chose the nine pairs to be tested. Each card pair was tested twice, once in each series of nine pairs. We then randomly chose the sequence of the pairs within each series of nine card pairs. We also chose which replicate to take for each pair and the side on which to place the green card. This side was randomly chosen for the first series of nine card pairs, and sides were alternated for the following series of nine card pairs. This ensured that each set of 18 pair presentations presented absolutely no bias in card presentation.

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Competing interests

The authors declare no competing financial interests.

Author contributions

All authors contributed to collection of data and elaboration of experimental design; A.G., C.D. and D.G. developed the concept of the experiment and wrote the article.

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