Ultraviolet colour vision and ornamentation in bluethroats

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SUMMARY

Many birds see in the ultraviolet (300–400 nm), but there is limited evidence for colour communication (signalling by spectral shape independently of brightness) in this 'hidden' waveband. Such data are critical for the understanding of extravagant plumage colours, some of which show considerable UV reflectance. We investigated UV colour vision in female social responses to the male UV/violet ornament in bluethroats, *Luscinia s. svecica*. In an outdoor aviary at the breeding grounds, 16 females were each presented with a unique pair of males of equal age. In UVR (UV reduction) males, sunblock chemicals reduced only the UV reflectance and thereby the spectral shape (colour) of the throat ornament. In NR (neutral reduction) males, an achromatic pigment in the same base solvent (preen gland fat) was used for a corresponding but uniform brightness reduction. Both colour and brightness effects were invisible to human eyes, and were monitored by spectrometry. In 13 of the 16 trials, the female associated most with the NR male, a preference that implies that UV colour vision is used in mate choice by female bluethroats. Reflectance differences between one-year-old and older males were significant only in UV, suggestive of a UV colour cue in age-related mate preferences.

1. INTRODUCTION

The plumage reflectance of many birds extends into the ultraviolet (320–400 nm) (Finger & Burkhardt 1994). This part of the daylight spectrum is invisible to humans and other primates, but is perceived by many birds, certainly as brightness and possibly as a fourth (or even fifth) channel in colour vision (Goldsmith 1994). After a long history of human-subjective colour assessment in behavioural ecology (Endler 1990; Bennett *et al*. 1994), it is important to address the communicative role of UV in avian plumage signals, epitomes of sexual selection ever since Darwin (Andersson 1994). Given the still scarce data on bird visual systems, especially the spectral range, dimensionality and resolution of colour vision, this has its problems. For example, inference of UV communication from signal design alone is rarely warranted, as most plumage UV reflectance can be regarded as secondary ('passive') effects of biochromes (pigments and scattering structures) with their main chromatic effects among longer wavelengths (Andersson 1996). Neither have any 'invisible', and thereby likely adaptive, UV patterns (as in some flowers and butterflies; Silbergleid 1979) been demonstrated in birds.

Behavioural evidence of avian UV signalling and reception are therefore needed, especially for communication by colour as such (hue, chroma) in the UV. This requires that responses to spectral reflectance shape are studied while controlling for brightness (intensity), which is difficult to achieve when receiver sensitivity is unknown (Jacobs 1981). However, as an initial approach, one can at least control objectively for signal brightness under (i.e. the radiance reaching the receiving eye; see Endler 1990) natural light conditions. Such tests of plumage signalling have recently been pioneered; in laboratory experiments on pekin robins, *Leiothrix lutea* (Maier 1994) and zebra finches, *Taeniopygia guttata* (Bennett *et al*. 1996), females rejected males behind UV cut-off windows, strongly suggesting that birds are sensitive to UV in a mate choice situation. As no preference was detected when only achromatic (neutrally absorbing) windows were used, the responses also seemed to involve UV colour perception. In addition, female zebra finches discriminated against males with leg band configuration asymmetries restricted to the UV (Bennett *et al*. 1996), which provided unique evidence for UV pattern perception.

In the above plumage signalling experiments, however, the whole light environments of males were altered (entire plumage, background, etc.), comparable to swapping between coloured and neutral eyeglasses. This complicates the inference of intraspecific mate choice as opposed to effects of species recognition or simply preference for the most natural illumination (i.e. the artificial daylight of their own chamber).

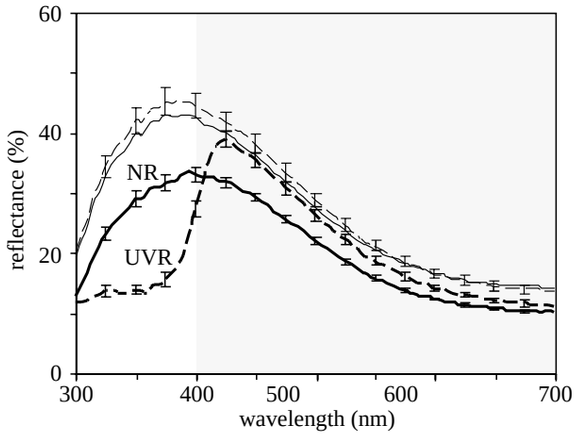

Figure 1. Spectral reflectance (mean ± s.e.) before and after UVR treatment (dashed lines) and NR (neutral brightness reduction) treatment (solid lines) for 11 experimental male pairs. Each of the 44 (4 × 11) spectra are in turn averages of five pre-treatment and three post-treatment scans, respectively, of the centre of the upper throat patch, removing the probe between each scan. To illustrate effects and contrasts within trial pairs, spectra in each trial were multiplied by a factor setting the pre-treatment reflectance of the NR male to the mean reflectance ($R_{365–400\,nm}$) of all pre-treatment scans. The shaded area denotes the (roughly) 400–700 nm spectral range of human vision.

We here explore UV colour vision under more natural signal and viewing conditions, and in response to a specific male colour ornament.

The bluethroat (*Luscinia s. svecica*) is suitable for such a study. First, the brilliant male throat patch, displayed to females during courtship, has a strong UV reflectance component (figure 1, thin lines), which might add considerable brightness and contrast to this signal compared to how it is seen by human eyes. Second, the bluethroat is a bird of mainly high altitudes and latitudes, displaying under open sky and most intensely early and late in the day (Merilä & Sorjonen 1994); these factors contribute to a skylight-dominated and thereby shortwave-biased light environment, suitable for UV and blue signals (Endler 1993). The bluethroat retina also has a large proportion of transparent cone oil droplets (Peiponen 1963), which are likely to transmit the shortest wavelengths. In accordance, behavioural tests have found bluethroats to discriminate accurately among the blue hues in the (human) Munsell colour atlas (Peiponen 1992). Taken together, bluethroat ecology and behaviour make it an excellent subject for a study of both extravagant plumage signalling and colour perception in the UV waveband.

Finally, previous studies have shown that male attractiveness in bluethroats decreases with experimental blackening of the throat patch (Johnsen & Lifjeld 1995), and that it increases with leg bands resembling the ornament colouration (Johnsen *et al.* 1997). This suggests that the throat ornament is important for mating success. Here we test whether the UV component of the signal also plays a role.

2. METHODS

The experiment was carried out on bluethroat breeding grounds in Heimdalen (61°25′N, 8°52′E, 1100 m.a.s.l.), Norway, 22–30 May 1996. We employed a previously successful method for aviary mate choice experiments in this species (Fiske & Amundsen 1997), and a new method based on sunblock chemicals to alter spectral reflectance shape specifically in the UV (Andersson 1998). During the course of the experiments, 30 bluethroat males and 21 females, recently arrived from migration and about to start pair formation, were mistnetted and held in individual, visually (sexes also acoustically) isolated cages. They were provided with water and mealworms *ad libitum*. Males were combined in 16 unique pairs (three of which reused one male from a previous pair; see below), matched with respect to age (1 or 2+ years old) and randomly assigned to either of two treatments of the blue throat patch: UVR (reduction of brightness only in the UV waveband) or NR (neutral i.e. spectrally uniform reduction of brightness).

The UVR treatment was obtained by smearing the throat plumage with a 40/60% (w/w) mixture of duck preen gland fat (CDC, Harrop Natural Fly dressing, Hoh Inc., St Anthony, USA) and UV-absorbing chemicals (50/50 w/w blend of Parsol 1789 and MCX, Givaudan-Roure, Surrey, England). The UVR treatment reduced throat reflectance in the range below 400 nm, but minimally affected reflectance in the human-visible wavelengths (figure 1). NR males were 'painted' with the same basic substance (CDC), but with the UV absorbents replaced by 2.5% Fe_2O_3 (Oxide black, Nitor AB, Norrtälje, Sweden), a uniformly absorbing black pigment. The purpose of the NR treatment was to reduce signal brightness by the same amount as in the UVR treatment, yet retain spectral reflectance shape across the 300–700 nm range (figure 1) so that UVR males deviated from NR males mainly in colour (hue, chroma) but not in brightness. If only the base solvent (CDC) had been used as control treatment, a significant result would not have allowed us to distinguish between responses to brightness and colour, a primary goal of this study.

The treatment effects were confirmed by reflectance spectrometry (see below). Mean (±s.d., $n = 11$) total reflectance, $R_{300–700}$, was reduced by 26.8% (±5.8) in UVR, and by 21.4% (±6.1) in NR males. As desired, when multiplied with the CIE D65 standard sunny daylight spectrum (CIE 1971) (an illuminant commonly used in vision research) this translated to nearly identical reductions in signal brightness of the two treatments (ΣR*D65; UVR: 18.8 ± 5.5%; NR: 20.0 ± 5.7%). In fact, due to natural random variation in pre-treatment contrasts, UVR males were on average 7.5% (±9.8) brighter (ΣR*D65) than NR males after treatment. Fortunately, this minor difference was in the conservative direction with regard to our result (see below). Colour and brightness changes were invisible to human eyes (authors plus three other observers). This was also confirmed by estimating treatment effects in terms of the human colour psychometrics brightness (L), hue angle (h_{ab}), and

chroma (C_{ab}) in the CIELAB colour space (CIE 1971), which in no case exceeded the normally least perceivable differences to humans (like between two neighbouring notations in a Munsell atlas).

Reflected radiance in the 365–700 nm spectral range was measured at ±0.5 nm resolution with a PS1000 spectrometer (Ocean Optics Inc., Dunedin, USA), with illumination from a regulated tungsten-halogen lamp, using a bifurcated quartz fibre optic probe held perpendicularly against the plumage surface (0°/0°) which yielded a 5 mm wide measuring spot. Reflectance is given in proportion to that of a Spectralon (Labsphere Inc.) white standard, scanned before the measurements. Reflectance 300–365 nm was extrapolated by the average of 20 museum specimens (Göteborg Nat. Hist. Museum), measured in the full 300–700 nm range with a combination of deuterium and tungsten illumination that we could not bring into the field (Andersson 1996). The museum spectra showed no increase in intraspecific variation or measurement error below 365 nm, and there was a strong correlation between reflectance at 375 nm and 325 nm ($r_s = 0.92$, $n = 20$, $p < 0.001$), why the extrapolation should be a good estimate of the reflectance shape in this region. For the treatment spectra (UVR and NR) we extrapolated the 300–365 nm section by using the extended absorption spectra of the coatings in this range, derived from a lab study of the manipulation technique (Andersson 1998).

Trials were performed in a 2 m high outdoor aviary with a surface area of 2.5 × 3 m. Within this aviary, a 3 × 1.5 m female compartment faced two 1.5 × 1 m male compartments, the latter visually isolated from each other by white, translucent glass fibre fabric. Remaining walls and roof were made of small-mesh fish netting. Males could thus not see each other, whereas the female could see both males (except when very close to either of the male compartments when she could not see the other male). A low mound of juniper branches allowed the female to avoid visual contact with males. Using a thin string, a 15 cm wide 'response zone' was delimited, within which the female was in visual contact with only one male. Time spent in this zone was scored as association with that male. A similar male zone, <30 cm from the net on his side, was used to restrict the sample of association to close contact between the sexes.

Behavioural observations were made from a hide 7 m away. From trial six (when we realized the opportunity given by invisible treatments), trials were made blindly, by letting a person other than the observer remove colour rings and release males into compartments, irregularly shifting left/right positioning between trials. Of the first five trials where male identity was known to the observer, two went in the opposite direction to the main result, so we are confident that no observer bias was introduced by including these in the final sample of 16 trials. During ⩾ 30 min acclimation, the female was visually isolated from the males by cardboard that was removed 10 min before observations. Trials lasted 30 min, during which female and male positions were point sampled every 10 s, i.e. 180 points. The number of points with female and male simultaneously in their respective zones was used as a measure of association. All males except two stayed close (<0.5 m) to the dividing net throughout the trial, showing interest in the female by displays, rushes at the net, etc.

Females spent more time in the response zone than predicted by chance (37 ± 22% vs. 10%, $t_{15} = 4.9$, $p < 0.001$), following males along the net, apparently trying to enter their cages, and one female solicited copulation. Five trials were rejected by the observer before treatment positions were revealed. In three of these, the female responded weakly (<10 recordings in response zone; 0, 0 and 5, respectively); these were repeated successfully with new females. In two trials one of the males spent most of the time trying to escape in the rear of his compartment; these trials were repeated with a new female and the elusive male replaced by a previously used male belonging to the same treatment group. No female (response) and no male-male combination (stimulus) was used more than once, but we present statistics also excluding the two partial pseudoreplicates.

3. RESULTS

Thirteen of the 16 different females associated more strongly with the NR than with the UVR male (binomial test, $p = 0.021$), with a strong within-trial skew of associations in favour of the NR males (figure 2; Wilcoxon matched-pairs signed-ranks test, $Z = -3.21$, d.f. = 15, $p = 0.0013$). This result was significant also when the two partial pseudoreplicates (each reusing one male from an earlier trial; see § 2) were excluded ($Z = -2.92$, d.f. = 13, $p = 0.0035$). Males were of equal age within stimulus pairs (2+ years in ten trials, and yearlings in six trials), and there was no effect of compartment (Wilcoxon $Z = -0.18$, d.f. = 15, $p = 0.85$).

As signal brightness was similar (on average slightly lower; see § 2) in NR males compared with UVR males, a discrimination based on UV colour, and not

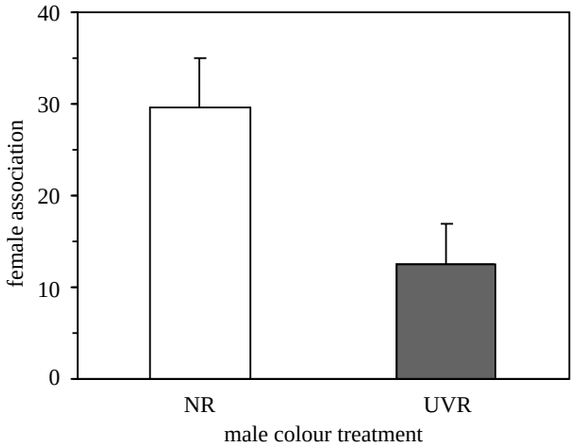

Figure 2. Association of female bluethroats with males of the two treatment groups; NR, neutral brightness reduction, and UVR, ultraviolet reduction. Female association is presented as mean ± s.e. of the number of point samples (10 s interval for 30 min) that females were recorded in the response zone close to either of the two males (see text).

just overall brightness, is strongly suggested. Interestingly, two of the three 'reversed' associations (i.e. with the UVR male) were the only spectrometrically monitored trials in which the UVR male (because of the random initial variation) was more than 10% brighter than the NR male after manipulations. This might indicate that brightness differences can be of some importance, and needs further testing to tease apart from colour cues. For our main result here, however, the altered spectral reflectance shape seems likely to have been the main preference cue, thereby suggesting that bluethroat colour vision encompasses also the UV waveband.

4. DISCUSSION

The results of this study provide behavioural evidence for bluethroat colour vision in the UV. This is probably achieved by a UV receptor at about 370 nm that has consistently emerged in studies of avian visual physiology and behaviour (Bennett & Cuthill 1994). Presumably, this receptor is wired to a tetrachromatic colour space, like that of goldfish (Neumeyer 1992), but this remains to be confirmed. Wavelength-dependent responses to UV without a colour sensation is one alternative interpretation (Goldsmith 1994). Neither can we completely rule out a response to achromatic brightness in our results, either from a preference for slightly duller NR males, or from a very strongly UV-biased spectral sensitivity that could render the NR males perceptually brighter in spite of our objective control. But along with other evidence for birds (Bennett *et al*. 1996; T. Goldsmith, personal communication), and other vertebrates with UV receptors (Tovee 1995), UV colour vision seems to be the most reasonable interpretation of our results.

Further work on bluethroats will reveal the characteristics of this UV colour perception, an exciting possible extension of the previously shown improvement of bluethroat hue discrimination towards shorter wavelength Munsell hues (Peiponen 1992). A shortwave or even UV-biased visual system in bluethroats would also be in accordance with their relatively high proportion of transparent retinal oil droplets (Peiponen 1963). Bluethroat vision may even parallel the two previously reported cases of high behavioural UV sensitivity in birds (Kreithen & Eisner 1978; Maier 1992), as opposed to the weak electrophysiological response to UV found in a number of passerine eyecups (Chen & Goldsmith 1986). Although retinal neurobiology provides important clues and constraints, behavioural data are needed to understand UV communication in birds (Goldsmith 1994).

The natural UV/violet hue that seems to be preferred by female bluethroats might represent a crude binary cue for species recognition and hybridization avoidance. However, as there is no Palearctic candidate for confusion with a bluethroat male, and since males with blackened throat patches also obtain mates (Johnsen & Lifjeld 1995), it seems likely that the UV/violet ornamentation is favoured by intraspecific female choice. Frequent extra-pair fertilizations (Krokene *et al*. 1996) and occasional polygyny (Peiponen 1960; Järvinen & Pietiäinen 1983; Johnsen & Lifjeld 1995) indeed suggest sexual selection in this species.

In addition, we found a natural ornament variation that might indicate a role of UV in age-related preferences: Older (2+ years) males ($n = 16$) had a 12.6% stronger UV reflectance ($\Sigma R_{300–400}$) than one year old males ($n = 8$) ($t_{22} = 2.45$, $p = 0.023$), but there was no significant difference in the human-visible spectrum ($\Sigma R_{400–700}$) ($t_{22} = 1.25$, $p = 0.22$). In addition, adopting human CIELAB (CIE 1971) colour psychometrics, older males had a significantly more short-wave (violet) human-perceived hue (hue angle, $h_{ab} = 272.8°$ vs. 268.6°, $t_{22} = 2.3$, $p = 0.032$). This hue difference in the violet-indigo range is very slight by human standards, but the difference may be perceivable in bluethroat colour space. Indeed, if a UV-encompassing human colour space is simulated by moving the CIE colour matching functions -80 nm, the hue difference between old and young males is twice as large, 9.3° ($t_{22} = 2.1$, $p = 0.048$). Ultraviolet hue and brightness might thus reveal male age (and thereby perhaps quality) to the choosy female, which completes a tentative scenario for sexual selection in the UV.

In conclusion, the female response to the spectral reflectance shape in UV of the male ornament suggests that UV sensitivity is an integral part of bluethroat colour vision, rather than performing some isolated visual task. We have also, for the first time, provided an indication of both ornament elaboration and mate choice operating in the UV waveband, beyond the range of human vision. Darwin (1871, p. 74) wrote that 'every one knows how splendid are the tints of birds'. It is becoming clear that there is even more splendour in bird plumages than meets our eyes.

We thank Malte Andersson, Andrew Bennett, John Endler, Tim Goldsmith, Frank Götmark, the 'green table' group at Burke Museum, Seattle, and several anonymous referees for helpful comments on various versions of this manuscript. We also thank Bjørn Bjerke, Erik Edvardsen, Elisabet Forsgren, Bo Hansen, Jo Heggset, Arild Johnsen, Jan T. Lifjeld, Percy A. Rohde and Jonas Örnborg for assistance in capturing and housing the birds. Spectroradiometry equipment was funded by the Swedish NSRC.

REFERENCES

Andersson, S. 1998 Testing the functional ecology of UV with human sunblock. *Funct. Ecol.* (Submitted.)

Andersson, M. 1994 *Sexual selection*. Princeton University Press.

Andersson, S. 1996 Bright ultraviolet coloration in the Asian whistling-thrushes. *Proc. R. Soc. Lond.* B **263**, 843–848.

Bennett, A. T. D. & Cuthill, I. C. 1994 Ultraviolet vision in birds: what is its function? *Vision Res.* **34**, 1471–1478.

Bennett, A. T. D., Cuthill, I. C. & Norris K. J. 1994 Sexual selection and the mismeasure of color. *Am. Nat.* **144**, 848–860.

Bennett, A. T. D., Cuthill, I. C., Partridge, J. C. & Maier, E. J. 1996 Ultraviolet vision and mate choice in zebra finches. *Nature* **380**, 433–435.

CIE 1971 *Colorimetry: official recommendations of the International Commission on Illumination (CIE)*. Paris: Bureau central de la CIE.

Chen, D. & Goldsmith, T. M. 1986 Four spectral classes of cone in the retinas of birds. *J. Comp. Physiol.* A **159**, 473–479.

Darwin, C. 1871 *The descent of man, and selection in relation to sex*. London: John Murray.

Endler, J. A. 1990 On the measurement and classification of colour in studies of animal colour patterns. *Biol. J. Linn. Soc.* **41**, 315–352.

Endler, J. A. 1993 The color of light in forests and its implications. *Ecol. Monogr.* **63**, 1–27.

Finger. E. & Burkhardt, D. 1994 Biological aspects of bird coloration and avian colour vision including the ultraviolet range. *Vision Res.* **34**, 1509–1514.

Fiske, P. & Amundsen, T. 1997 Female bluethroats prefer males with symmetric colour bands. *Anim. Behav.* **54**, 81–87.

Goldsmith, T. H. 1994 Ultraviolet receptors and color vision—evolutionary implications and a dissonance of paradigms. *Vision Res.* **34**, 1479–1487.

Jacobs, G. H. 1981 *Comparative color vision*. New York: Academic Press.

Järvinen, A. & Pietiäinen, H. 1983 The bluethroat *Luscinia s. svecica* population at Kilpisjärvi, Finnish Lapland: density, habitat selection, age structure and nesting success in 1981. *Proc. 3rd Nordic Congr. Ornithol.* 1981, 189–194.

Johnsen, A. & Lifjeld, J. T. 1995 Unattractive males guard their mates more closely. An experiment with bluethroats (Aves, Turdidae: *Luscinia s. svecica*). *Ethol.* **101**, 200–212.

Johnsen, A., Lifjeld, J. T. & Rohde, P. A. 1997 Coloured leg bands affect male mate-guarding behavior in the bluethroat. *Anim. Behav.* **54**, 121–130.

Kreithen, M. L. & Eisner, T. 1978 Ultraviolet light detection by the homing pigeon. *Nature* **272**, 347–348.

Krokene, C., Anthonisen, K., Lijfeld, J. T. & Amundsen, T. 1996 Paternity and paternity assurance behaviour in the bluethroat, *Luscinia s. svecica*. *Anim. Behav.* **52**.

Maier, E. J. 1992 Spectral sensitivities including the ultraviolet of the passeriform bird *Leiothrix lutea*. *J. Comp. Physiol.* A **170**, 709–714.

Maier, E. J. 1994 Ultraviolet vision in a Passeriform bird—from receptor spectral sensitivity to overall spectral sensitivity in *Leiothrix lutea*. *Vision Res.* **34**, 1415–1418.

Merilä, J. & Sorjonen, J. 1994 Seasonal and diurnal patterns of singing and song-flight activity in bluethroats (*Luscinia svecica*). *Auk* **111**, 556–562.

Neumeyer, C. 1992 Tetrachromatic color vision in goldfish—evidence from color mixture experiments. *J. Comp. Physiol.* A **171**, 639–649.

Peiponen, V. A. 1960 Verhaltenstudien am Blaukelchen *Luscinia s. svecica*. *Orn. Fenn.* **37**, 69–83.

Peiponen, V. A. 1963 Experimentelle untersuchungen über das farbensehen beim Blaukelchen, *Luscinia svecica* (L.) und Rotkelchen, *Erithacus rubecula* (L.). I. *Annls Zool. Soc. 'Vanamo'* **24**, 1–49.

Peiponen, V. A. 1992 Colour discrimination of two passerine bird species in the Munsell system. *Orn. Scand.* 23, 143–151

Silbergleid, R. E. 1979 Communication in the ultraviolet. *A. Rev. Ecol. Syst.* **10**, 373–398.

Tovee, M. J. 1995 Ultraviolet photoreceptors in the animal kingdom—their distribution and function. *Trends Ecol. Evol.* **10**, 455–460.

Received 15 July 1997; accepted 25 July 1997