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Blue tits are ultraviolet tits

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The blue tit (*Parus caeruleus*) has been classified as sexually monochromatic. This classification is based on human colour perception yet, unlike humans, most birds have four spectrally distinct classes of cone and are visually sensitive to wavelengths in the near-ultraviolet (300–400 nm). Reflectance spectrophotometry reveals that blue tit plumage shows considerable reflection of UV light. For example, the blue crest shows peak reflectance at wavelengths around 352 nm. Furthermore, the blue tit is sexually dichromatic for multiple regions of plumage, including the crest. Choice trials performed in the laboratory indicate that females prefer males with the brightest crests. This study has implications for both intra- and interspecific studies of sexual selection, as well as future classification of dichromatism, which should not ignore the possibility of variation in reflectance in the UV.

Keywords: sexual selection; sexual dichromatism; UV vision; spectral reflectance; female choice; blue tit

1. INTRODUCTION

A common form of sexual dimorphism is a difference in pattern or coloration known as sexual dichromatism. Birds are probably the most popular subject in studies of sexual dichromatism and sexual selection (Andersson 1994; Bennett *et al*. 1994) yet in virtually all cases, colour has been assessed by human observers, without consideration of the differences between human colour vision and the colour visual system of birds (Bennett *et al*. 1994).

Avian colour vision differs from that of humans in several fundamental ways (Bennett & Cuthill 1994). First, most birds are sensitive to near-ultraviolet wavelengths (300–400 nm) to which humans are blind (reviewed by Bennett & Cuthill 1994). Second, they have at least four spectrally distinct cone-types compared with our three (Bowmaker *et al*. 1997), implying that birds have the potential for tetrachromatic, if not higher-dimensional colour vision (Thompson *et al*. 1992). Third, avian cone-cells contain light-absorbing oil droplets which act as cut-off filters and reduce the overlap between cone spectral-sensitivities, compared with humans (Goldsmith *et al*. 1984; Partridge 1989; Bowmaker 1991). Consequently, a human description of bird coloration may be inadequate. In particular, species which appear monochromatic in the human visible spectrum may be dichromatic in the UV. This would have significant implications for both intra- and interspecific analyses of sexually selected traits.

The blue tit, *Parus caeruleus*, is typically monogamous and considered to be only very slightly dimorphic, with large overlap between the sexes both in morphometry and coloration (Perrins 1979; Svensson 1992). Hamilton (1990) proposed that sexual dichromatism may be more closely tied to extra-pair copulation than to choice of a social mate and blue tits exhibit relatively high levels of extra-pair paternity (17.9%: Møller & Birkhead 1994; 11–14%: Kempenaers *et al*. 1997). Females prefer extra-pair matings with high quality males (Kempenaers *et al*. 1992; Kempenaers *et al*. 1997), but how females assess male quality remains unclear. Size and song output may be important (Kempenaers *et al*. 1997) but, as in many birds, plumage characteristics may also play a role (Andersson 1994).

Burkhardt (1982), using UV photography, first indicated that blue tits might be UV reflective. In this study, and a companion paper by Andersson *et al*. (1998, this issue), sexual dichromatism in wild-caught blue tits is investigated by using reflectance spectrophotometry. We measured reflectance of multiple regions of plumage across the bird visible spectrum (300–700 nm) and used a molecular-sexing technique to determine the sex of individual blue tits in an unequivocal way. In addition, we did laboratory mate-choice trials to assess whether male plumage or morphology were likely criteria for female choice, whereas Andersson *et al*. (1998) examine patterns of pairing in the field.

2. METHOD

Blue tits were mist-netted and brought into captivity (1 February–22 March 1997) from capture sites in South England. They were housed individually under a natural photoperiod (changed weekly to match ambient photoperiod) at 18 °C. Their diet consisted of commercial insect mix, mealworms, dehusked sunflower seeds and peanuts. Water (containing vitamin supplement) was available *ad libitum*.

We measured plumage reflectance from live birds, 7 d after capture, sampling ten regions of plumage: the crest, primary coverts, top-of-tail, undertail, chest, back, white cheek, black throat, blue-black nape and white stripe above the beak (referred to as the white crown). Plumage was illuminated at 45° to the surface by a Zeiss CLX 500 xenon lamp and reflected light collected at 135° (90° to illumination) using a Zeiss MCS 500

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spectroradiometer, both parallel to the feather direction, with illumination from the proximal end. We took six measurements at randomly chosen locations within each region for each individual with a dark current before each measurement. In all, 1090 spectra were analysed. Reflectance was calculated relative to a Spectralon^{TM} 99% white standard for the wavelength range 300–700 nm. Measurements were done blind to the sex of the individual.

Blood samples were subsequently collected from the brachial vein of each individual, mixed with an equal volume of BLB (2% sodium dodecyl sulphate, 50 mM EDTA, 50 mM Tris.Cl pH 8) and stored at −70 °C. DNA was extracted and subjected to a modification of the bird sex identification technique of Griffiths *et al*. (1996). This method can identify two Chromo-Helicase-DNA binding (CHD) genes, one of which is unique to the female W sex chromosome, whereas the other is a positive control as it is situated on the Z sex chromosome (male ZZ, female ZW; Griffiths *et al*. 1998).

For eight out of ten regions, nine birds of each sex were used in the analysis. For the white crown and the black throat we measured five birds and six birds of each sex respectively, all caught on the same dates. We did principal components analysis (PCA) on spectra from each region separately. PCA transforms a large number of correlated variables (in this case, reflectance at 2.5 nm intervals) into a few orthogonal variables (the principal components, PCs) which summarize most of the original variation. Invariably, the first principal component (PC1) describes variation in mean reflectance, or brightness, as this forms most of the between-spectra variation (typically greater than 90%, cf. Hurlbert 1986; Endler & Thery 1996; Cronin *et al*. 1997). Subsequent principal components (PC2 and PC3) represent variation in spectral shape and are therefore indirectly related to hue and saturation (Endler 1990; Endler & Thery 1996). Male–female differences in PCA scores and the wavelengths of peaks in reflectance in the UV were analysed by using repeated measures ANOVA; PCA scores were also entered into a discriminant function analysis.

To assess whether male traits influence female mate-choice, we did laboratory mate-choice trials. A total of seven females were each given a choice of two, randomly selected males. Experimental birds had been unable to view members of the opposite sex since being brought into captivity. In addition, females were presented with males captured at a different location and therefore it is unlikely that they had encountered those males before capture.

Choice trials were done in a modification of the apparatus described in Bennett *et al*. (1996). This consisted of a central chamber, where food and water were available, and two arms ending in stimulus cages containing the male birds. Baffles between the central chamber and the arms of the apparatus ensured that the female test bird could only view one male at a time. The chamber was illuminated by ten equispaced Truelite fluorescent tubes (see Bennett *et al*. 1996). Females were separated from males by filters which were fully transparent across the bird visible spectrum (300–700 nm).

We gave each female 3 h acclimatization to the test apparatus, immediately before her experimental trial. Trials began when a male bird was placed in each of the two stimulus cages and ended 10 h later when all birds were removed and returned to their normal housing cages. The number of hops a female made in front of each of the stimulus cages was recorded using electronically monitored perches.

For each male we recorded four morphological variables which may influence female choice: mass, fat score (on a subjective scale from 0 to 5; Helms & Drury 1960), tarsus length (mean of five measurements from each tarsus) and degree of symmetry (measured as the difference in right and left tarsus length). In addition, we measured the spectral reflectance of eight regions of plumage (the crest, primary coverts, primary wing feathers, top-of-tail, undertail, chest, back, and black throat), 48 h after each experimental trial. We took four measurements within each region for each individual. Spectrophotometry procedures were otherwise identical to those described above. As in the previous analysis, we carried out a PCA on reflectance between 300 and 700 nm for each separate region and calculated the wavelength of peaks in reflectance in the UV.

Males were ranked according to the number of hops females made while viewing them. We then tested whether any of the morphometric and spectrophotometric variables varied with male rank. We did Wilcoxon one-sample tests on the differences in mass, fat score, tarsus length and symmetry between rank 1 and rank 2 males. The principal components derived from the spectral reflectance curves were entered into repeated measures ANOVA. For each plumage region we tested for an effect of rank on PC1, PC2, PC3 and wavelength of peak UV reflectance. In all analyses we adjusted the significance level by using the Dunn–Šidák method (Sokal & Rohlf 1995), to correct for the number of dependent variables.

3. RESULTS

(a) *UV reflectance*

Figure 1 shows mean spectral reflectance for the crest, chest, back, blue-black nape and white crown. All regions show some reflection in the UV. In particular the crest, rather than being a pure blue as we see it (with a peak between 400 and 480 nm), has a strong peak of reflectance in the ultraviolet (mean of all bird means = 352 nm; see figure 1*a*). Other 'blue' regions of plumage have a similar spectral shape with maxima in the UV. However, the blue-black nape has relatively low overall reflectance (figure 1*d*), and the upperside of the tail tends to yield flatter reflectance curves compared with the crest.

The yellow chest and olive-green back show very similar spectral reflectance (figure 1*b*, *c*), implying that their structure and pigmentation are closely related. Both regions show a prominent peak in reflectance in the ultraviolet, as well as a sharp increase around 500 nm. The white cheek and white crown (figure 1*e*), and the grey of the underside of the tail have relatively flat reflectance curves extending into the UV and presumably appear achromatic to birds as well as humans.

(b) *Sexual dichromatism*

Blue tits are significantly sexually dimorphic for five out of ten regions sampled: the blue crest, upperside-of-tail, back, blue-black nape and white crown. Apart from the white crown, *F*-ratios are greatest for PC2 or PC3, implying that it is in spectral shape that males and females differ most (table 1). The crest shows significant between-sex variation in PC2 and there is a significant difference in the wavelength of peak reflection (table 1), with male crests reflecting maximally at shorter wavelengths (mean = 349 nm; s.e. = 1.94) than female crests (mean = 355 nm; s.e. = 1.14). Sex differences are greater in the UV than in other regions of the spectrum, as illustrated by the plot of *F*-ratio against wavelength (figure

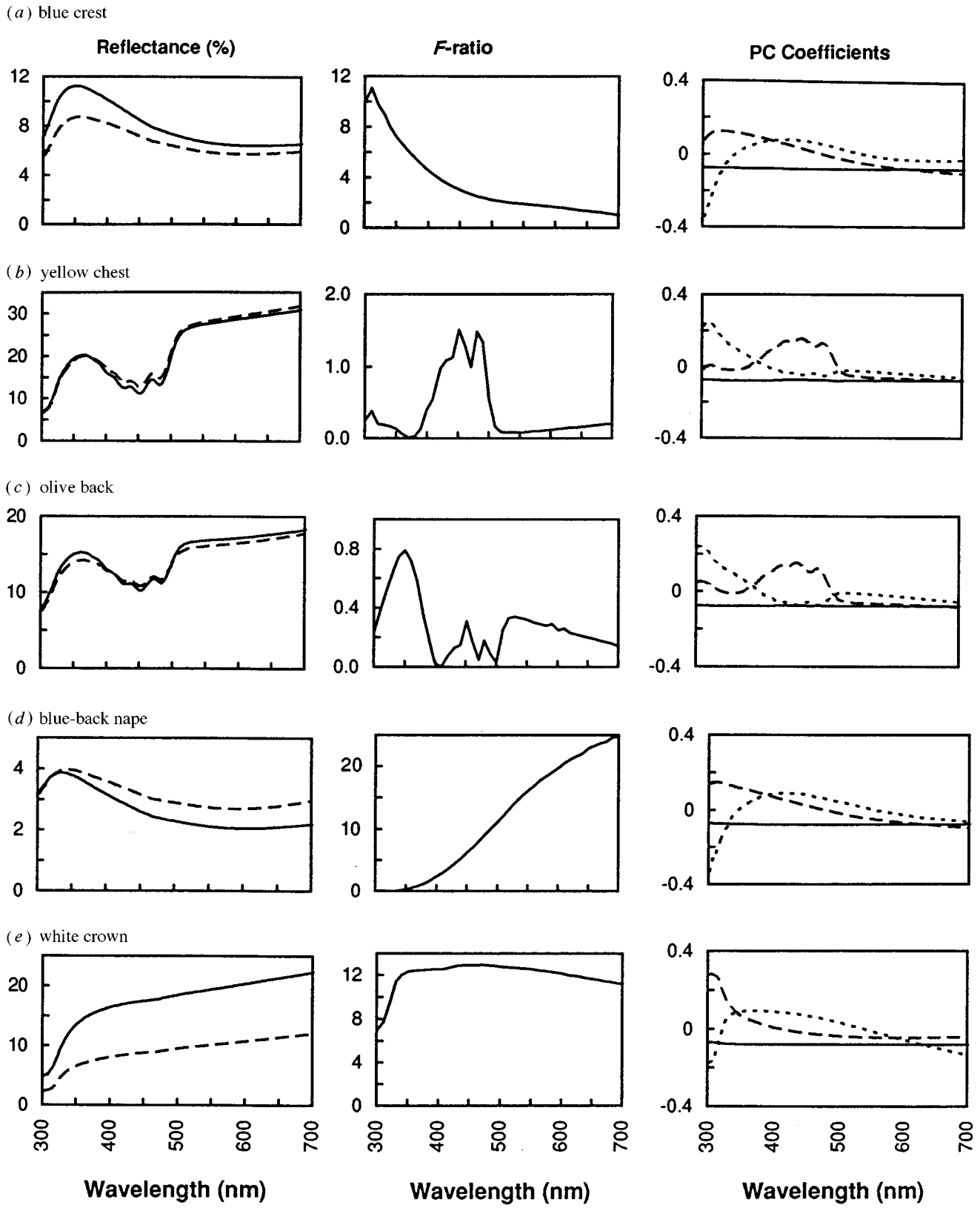

Figure 1. Plumage characteristics for five regions of blue tit plumage. Reflectance curves are the means of six measurements for nine (regions *a–d*) or five individuals (region *e*) of each sex. Male (indicated by a continuous line) and female (indicated by a broken line) spectral reflectance are significantly different in all regions except the chest (region *b*). *F*-ratios, calculated from univariate ANOVAs on the original reflectance values at 10 nm intervals between 300 and 700 nm, are provided to illustrate the regions of the spectrum for which between-sex differences in reflectance are greatest (cf. Brunton & Majerus 1995). Principal component coefficients illustrate the relation between PCs and wavelength; PC1 (indicated by a continuous line) is flat for all regions, indicating that it is likely to be correlated with achromatic brightness; the coefficients of PC2 (indicated by a broken line) and PC3 (indicated by a dotted line) vary with region and describe aspects of spectral shape and hence hue.

Table 1. *Sex differences in spectral reflectance of blue tit plumage*

(Values shown are *F*-ratios for the factor sex in univariate analysis of variance for each of the four variables, PC1, PC2, PC3 (derived from principal components analysis) and the wavelength of UV peak (if present). Values in parentheses under each principal component *F*-ratio are the percentage of between-spectra variation explained by that principal component. Analysis of each plumage region was done separately.)

		F-ratio (variation explained/%)				
region	d.f.	PC1	PC2		UV peak/nm	difference
crest	1, 12	3.36 (95.3)	9.69[a] (4.1)	8.23 (0.5)	9.32[a]	male spectra show steeper gradients; male UV peak at shorter wavelengths than females
coverts	1, 12	4.64 (98.2)	5.76 (1.5)	0.79 (0.1)	2.02	—
tail (upperside)	1, 12	2.70 (97.3)	33.94[b] (2.0)	0.02 (0.6)	0.24	male spectra show steeper gradients
tail (underside)	1, 12	4.61 (99.1)	0.18 (0.8)	0.144 (0.1)	—	—
chest	1, 12	0.17 (93.8)	1.01 (4.4)	6.75 (1.3)	4.80	—
back	1, 12	0.13 (97.3)	2.55 (2.1)	12.18[a] (0.5)	6.13	male spectra show steeper gradients
white cheek	1, 12	6.47 (98.4)	0.99 (1.0)	0.08 0.5)	—	—
blue-black nape	1, 12	7.91 (95.7)	21.33[b] (3.9)	2.17 (0.3)	6.62	male spectra show steeper gradients
white crown	1, 8	12.38[a] (97.9)	0.03 (1.8)	0.02 (0.3)	—	males brighter than females
black throat	1, 10	2.82 (97.6)	7.12 (1.8)	0.06 (0.3)	4.86	—

[a] Significant sex difference, $p < 0.01$.
[b] Significant sex difference, $p < 0.001$.

1*a*, cf. Brunton & Majerus 1995). A significant difference in PC2 is also found for the upperside of the tail and the blue-black of the nape (table 1). Reflectance of the back differs significantly between males and females in PC3. PC3 is related to reflectance below 400 nm (figure 1*c*). The white crown shows sex differences for PC1 indicating that males have brighter white crowns than females (table 1).

Discriminant function analysis showed 100% of both males and females to be correctly classified based on PCs 1–3 from the five sexually dichromatic plumage regions. Overall discrimination based solely on crest PCs was 67% (56% of males and 79% females correctly classified). Classification based on PCs from the crest, coverts and top-of-tail together, as used in subjective sexing in the field, was 89% correct. Svensson (1992) estimates that for experienced humans such field sexing is around 75% accurate, based on morphometry as well as human-visible coloration.

(c) *Mate choice*

Females showed a significant preference for males with the brightest crests. PC1 derived from crest reflectance spectra was significantly lower in rank 1 than rank 2 males ($F_{1,6}=14.88$, $p=0.008$). Crest PC1 was negatively correlated with mean reflectance (related to brightness; Endler 1990; cf. Bennett *et al*. 1997). Preferred males are therefore those with the brightest crests. No other spectrophotometric variables from any of the plumage regions showed a significant relation with female choice. Likewise, there was no difference in mass ($T=0.49$, $N=7$, $p=0.64$), fat score ($T=1.44$, $N=7$, $p=0.20$), tarsus length ($T=-1.32$, $N=7$, $p=0.24$) or tarsus symmetry ($T=0.82$, $N=7$, $p=0.44$) between the preferred or less preferred males.

4. DISCUSSION

Male and female blue tits not only differ in crest spectra (Andersson *et al*. 1998), but showed significant differences in spectra from the top-of-the-tail, back, white crown and blue-black nape. Discriminant function analysis on PCs from the five sexually dichromatic plumage regions, correctly classified 100% of birds by sex. Blue tits are therefore more sexually dichromatic than they appear to humans and there may be other species in which sexual dichromatism has been overlooked.

The reflectance spectra which underlie the colour of the blue tit crest peaked around 350 nm in the ultraviolet (figure 1*a*). The human-perceived blue is thus generated by merely the tail of a peak which would maximally stimulate an avian UV cone. Those passerines which have had their visual pigments characterized, typically have a UV cone absorbing maximally at 350–380 nm and a blue cone absorbing at 430–455 nm (Bowmaker *et al*. 1997). Preliminary data suggests the blue tit fits this pattern (N. Hart, unpublished data), so in avian terms the blue tit is misnamed and should perhaps be considered a 'UV tit'.

In contrast to our findings, crest reflectance spectra recorded by Andersson *et al*. (this issue) peaked above 400 nm. A possible explanation is the difference in the angle at which plumage reflections were collected. However, as the crest is not particularly iridescent such a large shift (*ca*. 80 nm) seems relatively unlikely. It is possible that reflectance may change with age and that our birds were of different ages, but Andersson *et al*. (1998) found no effect of age on spectral shape, only on brightness. There might therefore be a genuine difference between the two populations (see Svensson 1992).

Sexually dimorphic traits are particularly common signals in intersexual (and intrasexual) communication (Andersson 1994). In the blue-tit, male crests tended to be brighter than females', particularly in the UV, and there was significant between-sex variation in both spectral shape (as described by PC2) and the wavelength of peak reflectance (table 1). Mate-choice trials indicate that this region of plumage may play a role in female mate-choice as all females tested chose the male with the brightest crest (as measured by PCl for this region). This might result from a preference for older males since, in a Swedish population, brightness varied with age (Andersson *et al*., this issue). However, it also supports previous research showing that UV plumage reflections are important in intersexual signalling (Maier 1993; Bennett *et al*. 1996; Andersson & Amundsen 1997; Bennett *et al*. 1997; Hunt *et al*. 1997).

This is the first species shown to be sexually dichromatic in the UV but relatively monomorphic in the human visible spectrum. The work has implications for both intra- and interspecific studies of sexual selection. In particular, it is clear that classification based solely on human-perceived colours (which ignores the UV) may give misleading results.

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