

Sexual dichromatism in the yellow-breasted chat *Icteria virens*: spectrophotometric analysis and biochemical basis

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Sexual dimorphism or dichromatism has long been considered the result of sexual selection. However, for many organisms the degree to which sexual dichromatism occurs has been determined within the confines of human perception. For birds, objective measures of plumage color have revealed previously unappreciated sexual dichromatism for several species. Here we present an unbiased assessment of plumage dichromatism in the yellow-breasted chat *Icteria virens*. Chats exhibit yellow to orange throat and breast plumage that to the unaided human observer differs only subtly in color. Spectrophotometric analyses revealed that chat throat and breast feathers exhibited reflective curves with two peaks, one in the ultraviolet and one in the yellow end of the spectrum. We found differences in both the shape and magnitude of reflectance curves between males and females. Moreover, for feathers collected from the lower edge and middle of the breast patch, male plumage reflected more light in the ultraviolet and yellow wavelengths compared to females, whereas male throat feathers appeared brighter than those of females only in the ultraviolet. Biochemical analyses indicated that the plumage pigmentation consisted solely of the carotenoid all-*trans* lutein and we found that males have higher concentrations of plumage carotenoids than females. Feathers that were naturally unpigmented reflected more UV light than yellow feathers, suggesting a potential role of feather microstructure in UV reflectance.

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Sexual dichromatism has long been considered a strong indicator of the intensity of sexual selection (Darwin 1871, Andersson 1994). However, the existence or extent of sexual dichromatism has largely been described within the confines of the sensory capabilities of humans (Bennett et al. 1994, Cuthill et al. 1999). Birds often exhibit integumentary structures that reflect wavelengths invisible to humans (Bleiweiss 1994, Andersson 1996, Andersson and Amundsen 1997, Bennett et al. 1997, Hunt et al. 1998, Keyser and Hill 1999, Mahler and

Kempenaers 2002, MacDougall and Montgomerie 2003, Parker et al. 2003) and, not surprisingly, avian visual systems can detect ultraviolet (UV) wavelengths. Also, avian visual sensitivities and peak reflectance spectra of ornaments are often highly correlated, suggesting the possible importance of such ornaments as signals (Cuthill et al. 2000, Yokohama et al. 2000). Direct evidence for the importance of UV color as a sexual signal comes from mate choice studies both in the laboratory and the field showing female preferences for

males with more pronounced UV-reflecting ornaments (Andersson and Amundsen 1997, Andersson et al. 1998, Johnsen et al. 1998, Siitari et al. 2002).

Many of the avian species for which UV reflectance has been studied thus far are dichromatic both in the UV and visible spectrum (Andersson and Amundsen 1997, Bennett et al. 1996, Keyser and Hill 1999), but certain species that appear to be sexually monochromatic to humans may be dichromatic when viewed in the UV (e.g. blue tits, *Parus caeruleus* Hunt et al. 1998; Picui dove, *Columbina picui*, Mahler and Kempnaers 2002). Clearly, recognizing previously undetected sexual dichromatism is critical to understanding intra- and intersexual signals. Moreover, most studies of UV plumage dichromatism have focused on blue, short-wavelength coloration, where the UV component to plumage color exists as part of a single reflectance peak along with visible blue wavelengths (Andersson 1996, Andersson and Amundsen 1997, Hunt et al. 1998, Keyser and Hill 1999). Comparatively long-wavelength reflectance, as in green or yellow plumage, has been shown to exhibit separate spectral peaks, one in the visible range and one in the UV (Hunt et al. 1998, MacDougall and Montgomerie 2003, Parker et al. 2003).

Compared to simply documenting variation in plumage chromatism both within and between the sexes, less work has focused on the proximate mechanisms of plumage dichromatism. The proximate mechanisms of sexual dichromatism can be pigmentary or structural. Many birds exhibit red, orange, or yellow plumage based on carotenoid pigmentation (Fox 1976, Stradi 1998). Other species arrange the microstructure of feather tissues to scatter incident light and generate a structural based plumage dichromatism that is often blue or UV in color (Prum 1999, Prum et al. 2003). Certain birds exhibit only one of the aforementioned forms of sexual coloration (e.g. carotenoid pigmentation in the house finch, *Carpodacus mexicanus*; Hill 1992, Hill et al. 2002), whereas others may exhibit both (e.g. blue tit; Hunt et al. 1998).

UV reflectance in single peak spectral curves is almost certainly created by the same proximate mechanisms that generate the visible, short-wavelength portion of the peak (Andersson 1996, 1999). But the mechanisms underlying sexual differences in dual peak plumage coloration are unclear. To date few studies exist documenting the variation in dual peak reflecting plumage and fewer still examining the proximate mechanisms that produce these patterns.

Here, we investigate sexual differences in the spectral-reflectance patterns of yellow breast plumage in the yellow-breasted chat (*Icteria virens*, Family: Emberizidae, hereafter referred to as "chats"). Chats are socially monogamous Neotropical migrants that breed in edge habitats throughout North America (Dunn and Garrett 1997, Eckerle and Thompson 2001, Mays 2001). Both

male and female chats exhibit a bright lemon-yellow breast patch, sometimes tinged with orange, which extends from the throat to the abdomen, where white plumage predominates. Differences in plumage between male and female chats are, to the human eye, subtle. Male and female chats may exhibit differences that, while not apparent to the human eye, might be apparent to conspecifics. Given that UV reflectance has been observed in yellow plumage in other birds (Hunt et al. 1998, MacDougall and Montgomerie 2003, Parker et al. 2003), we suspected that a full and unbiased measure of plumage color might uncover previously unknown levels of dichromatism and therefore hint at potential targets of sexual selection in this species. Additionally, we conducted a preliminary investigation of the biochemical basis for color in the breast plumage of chats using conventional reverse-phase high-performance liquid chromatography (HPLC; e.g. Stradi et al. 1995).

Methods

General field methods

We studied chats at the Central Kentucky Wildlife Management Area, located 17-km southeast of Richmond in Madison County, Kentucky, USA from 1994–2000. The 680-ha area consists primarily of abandoned agricultural fields in various stages of succession. We delineated breeding territories by noting the locations of singing males and male-male interactions and captured adults by placing mist nets on active territories. Captured birds were fitted with a unique combination of three colored leg bands plus a numbered United States Fish and Wildlife aluminum band.

Sex of captured chats was determined by differences in lore plumage coloration, bill color, and the color of the mouth lining. During the breeding season, the lores of female chats are lighter (gray rather than black). In addition, male chats have a considerably darker lower mandible than females, and the mouth lining of females is pink while that of males is black (Pyle 1997, Dunn and Garrett 1997, Eckerle and Thompson 2001, Mays 2001). Behavioral observations of color-banded individuals also helped confirm sex since only males are known to sing (Dunn and Garrett 1997, Eckerle and Thompson 2001) and are more conspicuous in their movements compared to females (Mays 2001).

During the 1994 season, we determined the extent of the breast patch by measuring the proportion of yellow plumage, using a ruler, along three lines, one extending along the middle of the breast and two lines traversing each lateral side of the breast patch. All three lines extended from the base of the bill to the vent. The extent of the breast plumage is expressed as the mean percent of yellow plumage along all three lines.

During the 1998 and 1999 breeding seasons, we also collected feathers from chats for later analysis. Three feathers were collected from each of three regions along the medial line extending from the abdomen to the upper throat. We defined these three regions as upper (chin/upper throat), middle (center of the yellow breast plumage), and lower (just above where the yellow breast plumage ends and meets the white plumage of the abdomen). Upon collection feathers were stored in 1.5-ml micro-centrifuge tubes in the dark at room temperature.

Reflectance spectrography

Reflectance spectra of single feathers were determined using an Oriel reflectance spectrometer system (Thermo Oriel, Stratford, CT) with a sighting optic (Nikon UV-transmissive macro-lens; 105 mm), fixed image compact spectrograph (285–735 nm), Oriel Instaspec IV (1024 × 256 CCD), and a capture board installed on a desktop PC. Feathers were illuminated with a 250-watt stabilized xenon-arc light source (CVI Laser Corp., Albuquerque, NM) at approximately 45 degrees to the perpendicular. Feather spectra were measured against a flat black background with uniform, low reflectance across all spectra (<1% reflectance). Use of a macro-lens as a sighting optic for our measurements allowed us to carefully choose areas where there was no overlapping or split barbs. We only recorded spectrographic measures from areas where the feather barbs were aligned in parallel and nonoverlapping. For each feather a single reflectance curve was obtained by averaging 20 reflectance spectra at a sampling rate of 100 milliseconds. Reflectance spectra (285–735 nm) were calculated relative to a white standard (reflecting 99% of incident light) of Spectralon® (Labsphere, Inc., North Sutton, NH). While the spectrometer system measured reflectance between 285–735 nm, subsequent analyses only used reflectance spectra between 324–714 nm because it is unlikely that birds can detect wavelengths below 320 nm or into the infrared (above 700 nm, Cuthill et al. 2000). The spectrometer measured reflectance in 0.3 nm increments. Data was pooled into bins in 3 nm increments. Each spectral curve was divided into 131 variables each representing the mean percent reflectance of each bin between 324–714 nm. The mean reflectance for the three feathers collected from the throat/breast plumage for each individual was used in all subsequent analyses.

We first analyzed this multivariate dataset using principal component analysis (PCA). PCA is widely used in the analysis of reflectance spectra (Hunt et al. 1998, Cuthill et al. 1999, Grill and Rush 2000, Macedonia 2001, Mahler and Kempnaers 2002, MacDougall and Montgomerie 2003, Parker et al. 2003) and

provides a means of analyzing variation across the entire spectral curve rather than focusing only on specific segments. This analysis typically generates three statistically independent, orthogonal principle components (PC's). PC1 corresponds with brightness, the overall measure of light intensity at all wavelengths, while PC2 and PC3 capture variation in the shape of the spectral curve (Hunt et al. 1998, Grill and Rush 2000).

We analyzed sexual differences for each PC separately using ANOVA with feather position and sex as our dependent variables. To account for plumage variation across individual birds we included individual as a random term in the model. Additional separate PCA's were conducted for each of the three feather regions and these PC's were used in discriminant function analysis to test their ability to accurately classify a feather from each region as having been collected from a male or female. Discriminant function analyses generated canonical scores derived from PC's for each feather position and the ability of these scores to distinguish between males and females was assessed via classification matrices. Because we classified the same samples from which we derived the canonical scores, we obtained percent classification success for each analysis from a jackknifed classification matrix (McGarigal et al. 2000).

In addition to PCA and discriminant function analysis each analyzing variation across the entire spectral curve, we also examined the role of brightness at particular wavelengths in generating plumage dichromatism. Specific wavelengths were chosen for analysis based on regions of peak reflectance. Using the mean reflectance between 339–357 nm (UV) and the mean reflectance between 552–570 (visible yellow), each averaged across all three feather replicates, we compared the relative contribution of visible and UV wavelengths to sexual dichromatism for each feather position. Sexual differences in UV reflectance and visible yellow reflectance for each feather position using nonparametric tests. Discriminant function analyses were performed using SYSTAT version 9.01 (1998, SPSS Inc.) for Windows PC and all other statistical tests were performed using JMP version 4.0.2 (2000, SAS Institute Inc.) for Windows PC.

We produced spectral curves for feathers collected from throat/breast plumage for 39 females and 63 males. We obtained feathers for upper and middle regions but not the lower region for one female, and as a result this female was omitted from the PCA and discriminant function analyses but was included in comparisons of UV versus yellow reflectance.

Biochemical analyses

To examine the biochemical basis of chat plumage color, we subjected a subset of feathers (males $n = 15$; females $n = 6$; all collected from the middle region of the breast

plumage) to pigment extraction via conventional thermochemical methods (Hudon and Brush 1992) followed by analysis using HPLC (Stradi et al. 1995). Feathers were first sequentially washed in 1 ml ethanol and 1 ml hexane and dried for 30 min in a 65°C oven. We trimmed pigmented barbules from the feathers into a 9-ml glass tube and weighed portions to the nearest 10^{-6} g with an electronic balance (Mettler-Toledo AG245, Greifensee, Switzerland). We added 1 ml acidified pyridine (3 drops HCl in 50 ml pyridine) to the feathers, capped the tube with argon, and incubated the solution for 3 hr at 95°C. After cooling to room temperature, 1 ml distilled water and 5 ml of hexane:methyl-tert-butyl-ether (1:1, v/v) were added to the tube. We shook the mixture for 2 min and centrifuged the suspension for 3 min at 3000 rpm. The supernatant (colored upper phase) was transferred to a clean tube and evaporated to dryness under a stream of nitrogen. The remaining pigment residue was resuspended in 200 μ l of HPLC mobile phase (acetonitrile:methanol, 1:1, v/v) prior to HPLC analysis. 50 μ l of each sample was injected into a WatersTM 717plus Autosampler HPLC (Millipore Corp., Milford, MA) fitted with a Develosil RPAqueous RP-30 HPLC column (250 \times 4.6 mm I.D.; Nomura Chemical Co., Ltd., Japan) and an Eppendorf TC-50 column heater (set at 32°C). The pigments were analyzed isocratically using an HP 1050 Series Isocratic Pump at a constant flow rate of 1.2 ml/min and detected at λ_{max} with a WatersTM 996 photodiode array detector. We confirmed the identity of plumage carotenoids by comparing retention times (t_R) and absorbance maxima (λ_{max}) to authentic reference pigments donated by Roche Vitamins Inc. (Parsippany, NJ). Carotenoid concentration was determined by comparing HPLC peak areas, integrated by MillenniumTM software (version 2.1), to those for an internal standard (canthaxanthin, Roche Vitamins Inc., 1.5 μ g/ml, t_R : 15.8 min, λ_{max} = 470 nm) that we added prior to pigment extraction.

To learn more about how feather carotenoids contributed to plumage color, we also obtained reflectance curves for both naturally unpigmented and pigmented barbules within the same feather ($n = 16$). For some feathers we obtained reflectance data for white and yellow regions of the same feather both prior to and following depigmentation ($n = 10$). Because our spectral analyses of depigmented feathers were performed well after our initial data collection and at a different institution, the same spectrophotometer was not available. The use of different methodologies to obtain our initial measures of sexual dichromatism and later spectra from depigmented plumage warrants some caution in making quantitative comparisons between these two datasets.

These data were collected using an Ocean Optics S2000 spectrophotometer (range 250–880 nm) equipped with ultraviolet (deuterium) and visible (tungsten-halo-

gen) lamps and a bifurcated 200 micrometer fiber-optic probe (Dunedin, Florida, USA). The fiber-optic probe both provided illumination and obtained light reflected from the sample. Feathers were arranged on a piece of black construction paper in groups of three and measurements were taken from areas where barbules were aligned to completely cover the black background. The fiber-optic detection/illumination probe was held at a 90-degree angle to the sample surface and ambient light was excluded with a block sheath that held the probe in place. This set-up provided a reading area of approximately 1-mm. All reflectance curves were generated relative to a white standard (WS-1, Ocean Optics, Dunedin, Florida, USA). We used OOIBase software to generate average curves based on 20 spectra measured sequentially. We repeated this three times for each region of the feather (distal pigmented barbules and proximal unpigmented white barbules) each time lifting and repositioning the probe. These three measures were again averaged to get a curve for each region of the feather. Curves were arranged by averaging the percent reflectance in 3.25 nm bins beginning at 320.2 nm and ending at 770.08.

Results

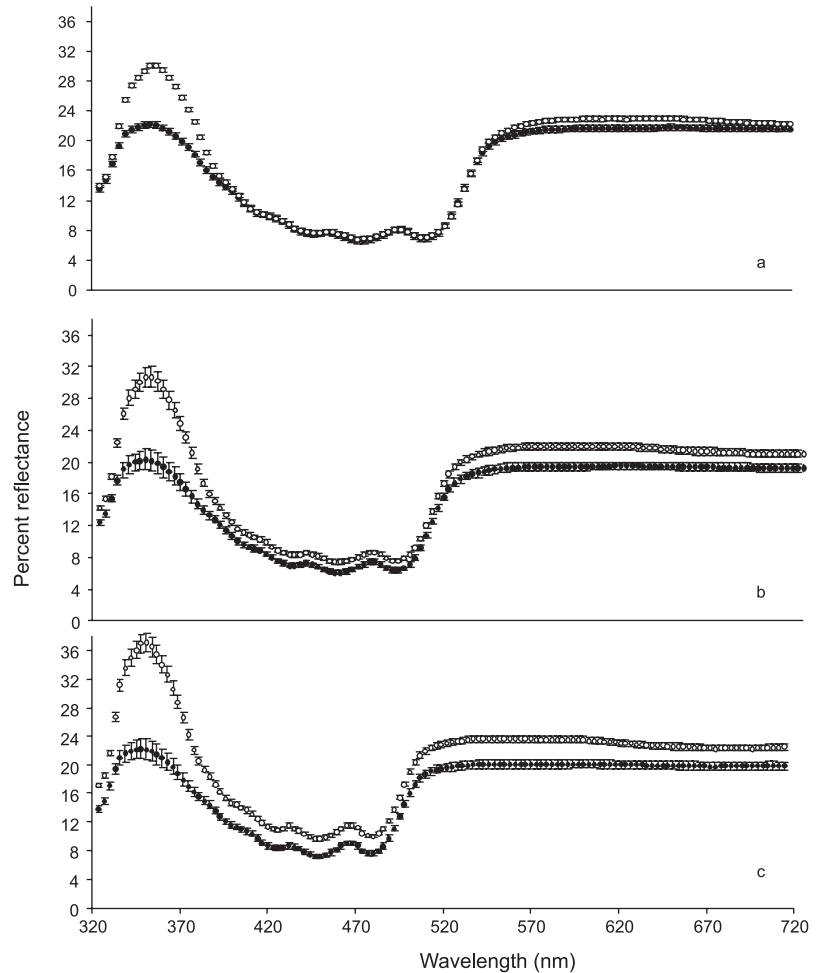
Spectrographic analyses

Reflectance curves (324–714 nm) varied significantly between sexes (Fig. 1). PCA generated three PC's each with an eigenvalue > 1 and these first three PC's explained over 99% of the variation in reflectance curves. Analysis of these principal components show significant differences between plumage characters for males and females, but the exact nature of the dichromatism differed with feather position.

Male plumage was brighter on average compared to that of females (PC1 males = 0.12 ± 0.10 ; female PC1 = -0.20 ± 0.13 ; ANOVA, $F_{1, 197} = 3.55$, $P = 0.06$). The effect of feather position was highly significant (ANOVA, $F_{2, 197} = 19.78$, $P < 0.0001$) with upper feathers being brighter on average than plumage collected from the middle and lower regions of the breast (lower PC1 = -0.16 ± 0.10 ; middle PC1 = -0.16 ± 0.10 ; upper PC1 = 0.32 ± 0.10). There was no interaction effect of sex and feather position for PC1 (ANOVA, $F_{2, 197} = 0.83$, $P = 0.44$).

In addition to differences in feather brightness, feathers also varied significantly in terms of spectral shape. PC2 differed significantly between the sexes (ANOVA, $F_{1, 197} = 4.43$, $P < 0.05$) and among feathers collected from different positions (ANOVA, $F_{2, 197} = 104.45$, $P < 0.0001$). There was a significant interaction effect of sex and feather position (ANOVA, $F_{2, 197} = 16.01$, $P < 0.0001$). PC3 differed significantly between the sexes (ANOVA, $F_{1, 197} = 4.91$, $P < 0.01$) and across feathers collected from different positions (ANOVA,

Fig. 1. Mean reflectance curves (± 1 SE) for males (open circles) and females (closed circles) for feathers collected from the chin/throat (a), the middle of the breast plumage (b), and the caudal end of the breast plumage where the yellow plumage (c) meets the white plumage of the abdomen. Lower feathers, $n = 38$ females, $n = 63$ males; middle and upper feathers, $n = 39$ females, $n = 63$ males each.



$F_{2, 197} = 26.24$, $P < 0.0001$) but there was no interaction effect of feather position and sex for PC3 (ANOVA, $F_{2, 197} = 2.23$, $P = 0.11$). We also included individual as a random term in the model. For all three PC's there were highly significant effects of individual (PC1, ANOVA, $F_{100, 197} = 3.93$, $P < 0.0001$; PC2, ANOVA, $F_{100, 197} = 1.73$, $P < 0.001$; PC3, ANOVA, $F_{100, 197} = 24.36$, $P < 0.0001$).

Discriminant function analysis revealed that plumage PC's were successful at probabilistically separating feathers according to sex. Successful assignment of feathers to the sex from which they were collected was consistent across different feather positions (overall percent correct: lower 74%; middle 72%; upper 69%). However, for all three feather positions, female feathers were on average 21% more likely to be assigned to the sex from which they were collected (sex-specific percent correct: lower-females 84%, lower-males 68%; middle-females 87%, middle-males 63%; upper-females 84%, upper-males 60%).

We also assessed the relative importance of yellow versus UV reflective peaks by focusing specifically on sexual differences in those segments of the spectrum. Several of these values were non-normally distributed; therefore we compared males and females using non-parametric tests. Within an individual, mean percent reflectance in the UV (339–357 nm) and yellow wavelengths (552–570 nm) were significantly correlated with one another (Fig. 2). For all feather positions the correlation between UV and yellow reflectance was stronger for females (Spearman rank order correlation, r_s ; lower feathers, $r_s = 0.90$, $P < 0.001$, middle feathers, $r_s = 0.64$, $P < 0.01$, upper feathers, $r_s = 0.90$, $P < 0.001$) than for males (lower feathers, $r_s = 0.65$, $P < 0.01$, middle feathers, $r_s = 0.53$, $P < 0.01$, upper feathers, $r_s = 0.51$, $P < 0.01$). Significant sexual differences were found for mean percent reflectance (brightness) for both UV and yellow wavelengths for lower feathers. Likewise, middle feathers differed significantly for both UV and yellow wavelengths. However, upper feathers differed in

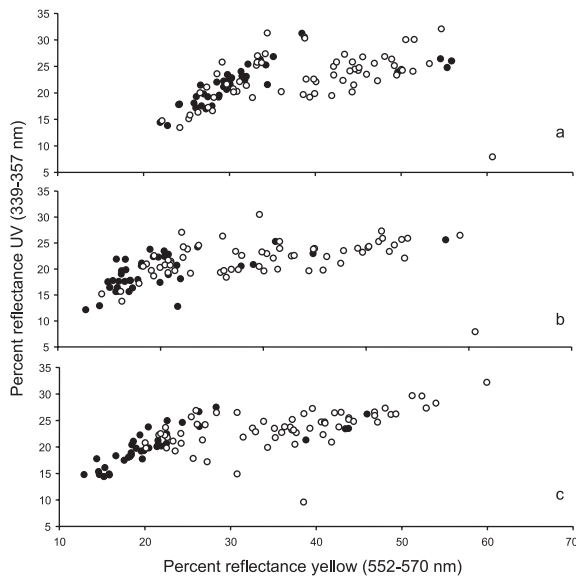


Fig. 2. Scatter-plots of mean reflectance UV (339–357 nm) and yellow (552–570 nm) for upper feathers (a), middle feathers (b) and lower feathers (c), and open circles represent feathers collected from males and closed circles are female feathers. Lower feathers, $n = 38$ females, $n = 63$ males; middle and upper feathers, $n = 39$ females, $n = 63$ males each.

UV but showed a nearly significant trend for males to exhibit brighter plumage in yellow wavelengths (Table 1).

While the breast patch color did vary significantly between males and females, the extent of the breast patch was comparatively sexually monomorphic. Males tended to exhibit larger breast patches with breast patches extending further posteriorly, but this difference was not statistically significant (females, mean-bar ± 1 SE = $51.6 \pm 0.02\%$, $n = 34$; males, mean-bar ± 1 SE = $52.5 \pm 0.02\%$, $n = 30$; $t = 1.83$, $P = 0.07$).

Mechanism of plumage color

Using the HPLC mobile phase, we identified a primary peak comprising over 60% of all eluted products in male and female feather samples. This pigment eluted at 10.5 min and absorbed light maximally at 445.4 and 474.5 nm, with some absorbance of short wavelengths

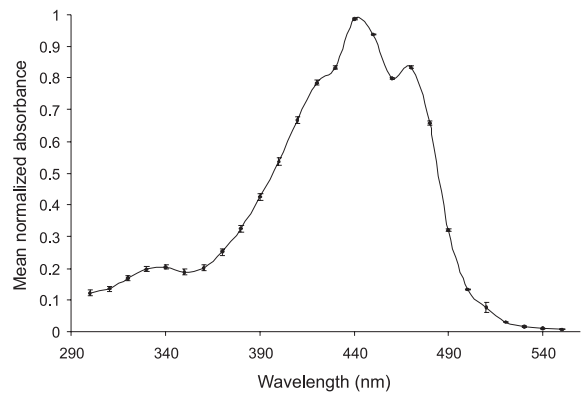


Fig. 3. Mean normalized absorbance of lutein products (± 1 SE) dissolved in ethanol that were thermochemically extracted from the yellow breast feathers of chats ($n = 21$). Data were collected using a Bausch and Lomb Spectronic 1001 spectrophotometer at 10 nm increments from 300–550 nm.

(< 360 nm) but considerably less above 500 nm (Fig. 3). By comparison to a series of external, authentic standards, we determined that this carotenoid in male and female chat plumage was consistent with all-*trans* lutein. Among all the birds in our analysis, all-*trans* lutein was present at an average (\pm SE) concentration of 1.06 ± 0.08 mg/g pigmented feather barbule, with all-*trans* lutein concentrations being significantly greater in the breast feathers of males compared to those of females (Wilcoxon two-sample test, $Z = -2.84$, $P < 0.01$; Fig. 4).

The t_R and λ_{max} values for the remaining, 4 small peaks did not match our other reference pigments, so we decided to compare the retention times and absorbance maxima of these unknown carotenoids to those for various lutein isomers (Bernstein et al. 2001, Dachtler et al. 2001, Negro et al. 2001). In fact, these peaks were consistent with 4 *cis* isomeric forms of lutein (λ_{max} and t_R in parentheses): 9-Z (331.4, 441.8, and 467.2 nm, 12.3 min), 9'-Z (same λ_{max} values as 9-Z, 12.7 min), 13-Z (334.9, 441.8, and 470.9 nm, 15.1 min), and 13'-Z (same λ_{max} values as 13-Z, 15.8 min) lutein. Because *cis-trans* isomerization is a possible outcome of thermochemical procedures (Subagio et al. 1999), we subjected our authentic, all-*trans* lutein standard (Roche Vitamins Inc.) to the same pyridine/heat treatment performed on the feathers. Whereas no *cis* isomers were evident prior

Table 1. Male-female comparisons in mean reflectance (± 1 SE) in the UV (339–357 nm) and visible yellow wavelengths (552–570 nm) for upper, middle and lower feathers (Mann-Whitney U test).

		Male	Female	n_{male}	n_{female}	P-value
Upper	UV	29.01 ± 1.14	21.79 ± 1.33	63	39	$P < 0.001$
	Yellow	22.88 ± 0.57	21.62 ± 0.58	63	39	$P = 0.08$
Middle	UV	29.63 ± 1.16	19.86 ± 1.05	63	39	$P < 0.001$
	Yellow	21.89 ± 0.44	19.35 ± 0.54	63	39	$P < 0.001$
Lower	UV	35.74 ± 1.25	21.90 ± 1.32	63	38	$P < 0.001$
	Yellow	23.59 ± 0.45	20.09 ± 0.57	63	38	$P < 0.001$

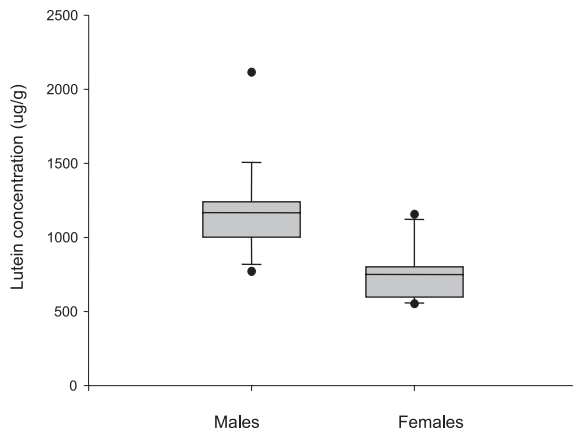


Fig. 4. Male-female comparisons in lutein concentration measured as μg of lutein per gram of feather. Box plots are shown as medians with 10%, 25%, 75% and 90% percentiles. Males $n = 15$, females $n = 6$.

to treatment, *cis* isomers appeared in the treated standard at the same retention times and in the same peak-area ratio (ca. 40% of total) as in chat feathers. From these data we conclude that only all-*trans* lutein (hereafter referred to as lutein) is present in chat plumage and that these geometrical isomers are artifacts formed during the extraction process.

We also used a much stronger HPLC solvent system (methylene chloride:methanol, 1:1, v/v) to determine if, in addition to lutein, any less polar carotenoids (e.g. β -carotene, β -cryptoxanthin) were present in chat plumage. We failed to detect any additional carotenoid products using this method.

Lutein concentration was significantly predictive of individual differences in mean percent reflectance in yellow wavelengths (552–570 nm, Spearman's $r = 0.59$, $P = 0.02$), and UV wavelengths (339–357 nm, Spearman's $r = 0.54$, $P = 0.04$). The same was true when we standardized yellow and UV reflectance levels separately to a mean of 0 and standard deviation of 1, to control for differences in the variability of the two measures.

Comparisons of reflectance curves from pigmented and unpigmented segments within a single feather show that the white unpigmented barbs display increased UV reflectance compared to the yellow, pigmented barbs (Fig. 5). Additionally, the feathers that we chemically depigmented with pyridine also display reflectance in the UV, but the magnitude and shape of the curve differs from that of naturally unpigmented plumage (Fig. 5). A repeated measure ANOVA of PC's derived from the variation of spectra taken from the white, naturally unpigmented parts of the same feathers ($n = 10$) both before and after treatment with pyridine revealed significant differences in PC1 ($P < 0.05$) and PC2 ($P < 0.01$) but not PC3 ($P > 0.05$).

Discussion

While the extent of the yellow plumage patch was similar for males and females, we found significant sexual dichromatism in the plumage of chats. The yellow patch exhibited dual-peak reflectance curves, characteristic of yellow plumage in other species (Hunt et al. 1998, MacDougall and Montgomerie 2003, Parker et al. 2003, Hill and McGraw 2004), with one area of peak reflectance in the UV and one in the visible yellow end of the spectrum. Chats showed nearly significant sexual differences in our estimates of overall plumage brightness (PC1), with males exhibiting brighter plumage than females and reflectance spectra showed significant intersexual variation in the shape of spectral curves (PC2 and PC3).

Discriminant function analysis, using PC's describing variation in the magnitude and shape of spectral curves, was successful at probabilistically distinguishing between male and female breast plumage. Feathers collected from females were more likely to be assigned to their correct sex than were feathers collected from males and sexual differences in plumage distinctiveness were greater for middle and upper feathers than for lower feathers.

Males showed significantly greater reflectance in both UV and yellow wavelengths for lower and middle feathers but differed significantly from females in the UV for upper feathers and showed a nearly significant trend for males to have brighter yellow upper plumage. This, along with a significant effect of feather position and a significant interaction between sex and feather position in the PCA generated across the entire spectral curve, indicates that throat/breast plumage in chats is non-uniform in regard to reflectance characteristics and therefore exhibit a pattern that is hidden from the unaided human observer.

Plumage color in chats is different from many other avian species since the UV reflectance occurs as a distinct peak from that found in the visible range. Unlike other species, UV reflectance in chats is therefore not merely due to spectral "spillover" of a single, short wavelength reflectance peak (Andersson 1996, Keyser and Hill 1999, Keyser and Hill 2000), but is more like that found in the yellow plumage of blue tits (Hunt et al. 1998), American goldfinches (*Carduelis tristis*, MacDougall and Montgomerie 2003, Hill and McGraw 2004), pine siskins (*Carduelis pinus*, Hill and McGraw 2004) and Kentucky warblers (*Oporornis formosus*, Parker et al. 2003). However, like Kentucky warblers (Parker et al. 2003) but unlike the dual-peak reflectance plumage in other species, chats appear to exhibit a non-uniform sexual dichromatism in the breast and throat plumage with some feathers being dichromatic for both UV and yellow spectra and some more dichromatic with regard to UV reflectance compared to visible spectra. This raises the possibility that UV and visible reflectance in chats and

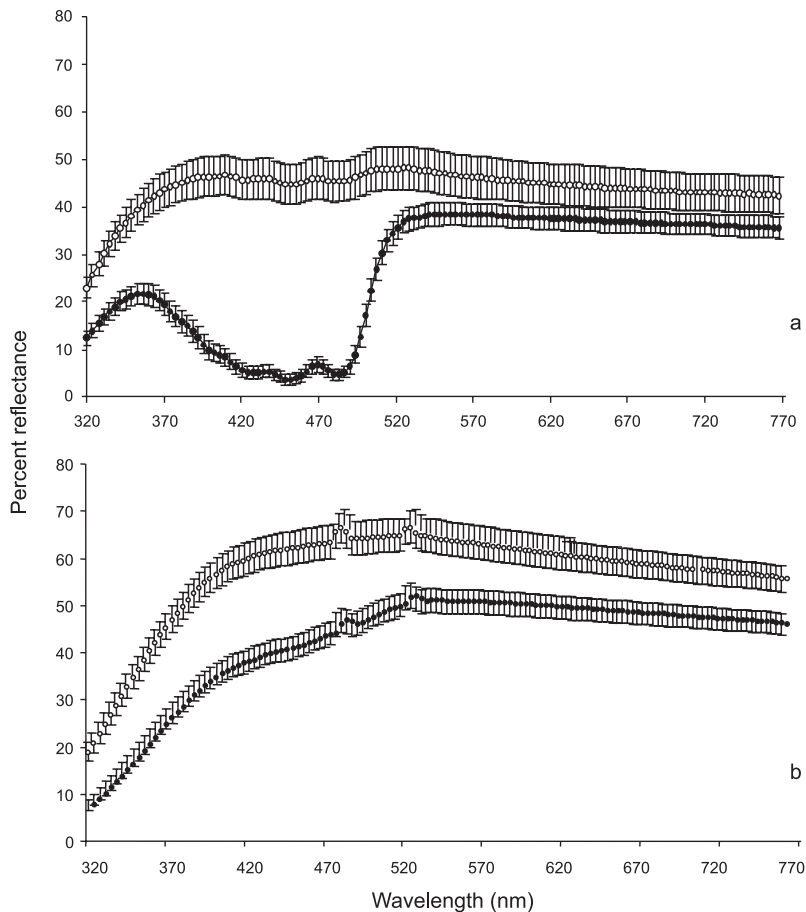


Fig. 5. Mean reflectance curves (± 1 SE) for the distal, pigmented, yellow barbs (closed circles) and naturally unpigmented, white barbs (open circles) of the same feather ($n = 16$, a) and mean reflectance curves (± 1 SE) for the distal (closed circles) and proximate barbs (open circles) of chemically depigmented feathers ($n = 10$, b).

other dual-peak plumage ornaments may have different proximate causes.

Yellow and red coloration in birds and many other organisms results primarily from carotenoid pigmentation (Stradi 1998) and chats are no exception. The yellow plumage of chats contained a single carotenoid, all-*trans* lutein. The carotenoid system of chat plumage is comparatively simple, as long wavelength plumage in most birds contains a complex mix of different carotenoid pigments (Stradi 1998). Plumage collected from male chats contained significantly higher concentrations of carotenoids than found in female plumage.

Carotenoids cannot be synthesized *de novo* and therefore must be acquired from the environment by consuming carotenoid-rich foodstuffs (Hill 1992, Olson and Owens 1998, Hill et al. 2002). Expression of carotenoid-based ornaments, therefore, is closely tied to an individual's ability to sequester resources and hence may act as an honest indicator of quality. In edge habitats in North America yellow/orange visible peaks in avian plumage often stem from the intake of carotenoids in late summer fruit from herbaceous shrubs and vines such as *Rubus* spp. and *Lonicera* spp. (Mulvihill et al.

1992). Chats on our study site consume large quantities of blackberries *Rubus allegheniensis* between July and August. This dietary change to a carotenoid-rich food source precedes a molt that is completed on the breeding grounds just prior to migration. Because chats spend the breeding season on all-purpose territories, obtaining the majority of their food within the confines of their territory boundaries (Eckerle and Thompson 2001, Mays 2001), variation in yellow plumage components can not only signal foraging ability but also territory quality during molt.

While the role of carotenoids, such as lutein, is well documented as the basis for long wavelength reflecting plumage in many birds, UV reflectance in avian plumage is relatively less studied especially in species with dual peak reflectance plumage. The UV component of plumage color is typically thought to result from structural properties of the feathers themselves rather than from pigmentation (Dyck 1976). Andersson (1996) measured UV reflectance in Asian whistling thrushes *Myiophonus* spp. and later characterized the feather microstructure using transmission electron microscopy (Andersson 1999). *Myiophonus* thrushes exhibit a

spongy feather structure, similar to that found in UV plumage in non-passerines (Dyck 1976, Andersson 1999), that produces UV reflectance by coherent scattering (Prum et al. 2003). Carotenoids are at first glance unlikely to be behind UV reflectance since there is some evidence that they absorb short-wavelength light (Gotz et al. 1999, Vershinin 1999). However, some carotenoids have been found not to absorb short wavelengths and by themselves may result in the UV peak found in green and yellow plumage (Burkhardt 1989, Finger and Burkhardt 1994). Lutein eluted from chat feathers in this study did absorb some short wavelength light, considerably more than is absorbed in the long wavelength visible end of the spectrum (above 500 nm). However, this absorbance was not very significant and lutein therefore reflects some UV and may contribute to UV reflectance variation across the sexes. Also, the precise manner in which carotenoids are bound to the feather keratin may alter their reflective properties (Stradi et al. 1995) and some carotenoid/keratin configurations could potentially exhibit UV reflective properties by expressing variation in the amount of UV absorbed.

The proximate mechanism creating UV reflectance in chat plumage may also be due to feather structure. The white, naturally unpigmented parts of chat feathers reflect UV wavelengths, as do the chemically depigmented parts of feathers. This suggests a non-pigment-based origin of UV reflectance in chat feathers. However, more work needs to be done on the proximate mechanism of dual peak reflective plumage before we can draw firm conclusions about the nature of UV color in these yellow feathers. Spectral data from depigmented feathers proved inadequate for addressing the proximate mechanism of color production since the depigmenting process significantly altered not only the reflectance curves for the pigmented parts of feathers but also for the unpigmented parts as well. A comparison of male and female feathers in terms of spongy microstructure, like that found in other highly UV reflective birds (Dyck 1976, Andersson 1999), would be a logical next step in elucidating the proximate mechanisms behind dual-peak reflecting plumage. If the UV component in dual-peak plumage results from intrinsic feather microstructure, while the long-wavelength component results from carotenoids, then dual-reflective plumage ornaments may be conveying multiple signals within a single feather. UV and yellow color components could potentially signal either different aspects of male quality or act as redundant signals by introducing additional sources of variation in male ornaments, thereby allowing females to better distinguish among males of differing quality (Møller and Pomiankowski 1993).

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