Journal of Zoology

Journal of Zoology. Print ISSN 0952-8369

Fluorescent and ultraviolet sexual dichromatism in the blue-winged parrotlet

A. S. Barreira[1], M. G. Lagorio[2], D. A. Lijtmaer[1], S. C. Lougheed[3] & P. L. Tubaro[1]

1 División Ornitología, Museo Argentino de Ciencias Naturales 'Bernardino Rivadavia', Ciudad Autónoma de Buenos Aires, Argentina
2 INQUIMAE Departamento de Química Inorgánica, Analítica y Química Física, Universidad de Buenos Aires, Ciudad Autónoma de Buenos Aires, Argentina
3 Department of Biology, Queen's University, Kingston, ON, Canada

Keywords
fluorescence; plumage coloration; UV reflectance; sexual dichromatism; Neotropical parrots.

Correspondence
Ana S. Barreira, División Ornitología, Museo Argentino de Ciencias Naturales 'Bernardino Rivadavia', Av. Ángel Gallardo 470, Ciudad Autónoma de Buenos Aires C1405DJR, Argentina.
Email: abarreira@macn.gov.ar

Editor: Andrew Kitchener

Received 27 October 2011; revised 19 April 2012; accepted 26 April 2012

doi:10.1111/j.1469-7998.2012.00931.x

Abstract

The presence of sexual differences in plumage coloration (sexual dichromatism) is frequent in birds. However, in many cases, humans cannot detect colour differences that are discernible to birds and it is therefore necessary to employ objective methods that contemplate the characteristics of the avian visual system for the study of plumage coloration. An understudied property of feather coloration is the occurrence of fluorescence, which has been described almost exclusively in parrots from the Eastern Hemisphere using non-objective methods and has been attributed to yellow pigments that are only present in psittacids. In this study, we explore fluorescence and sexual dichromatism through objective and quantitative methods in the plumage of a Neotropical species, the blue-winged parrotlet *Forpus xanthopterygius*. We measured plumage reflectance and fluorescence emission on museum skins using spectrophotometry and spectrofluorometry, respectively. The reflectance analysis revealed the presence of ultraviolet sexual dichromatism that adds to the differences in the visible range of wavelengths that are detectable by humans. The spectrofluorometric analysis showed that fluorescence is indeed present in this species, both in green plumage patches, where fluorescent pigments are presumably located, and in the blue rump of males, where colour is considered to be purely structurally based. The sexes differed in the intensity and wavelength of their fluorescence emission, representing the first finding of fluorescence sexual dichromatism in birds.

Introduction

Sexual dichromatism (i.e. the presence of colour differences between sexes) is a common form of sexual dimorphism in birds that typically involves the presence of plumages that are colourful in males but dull in females. It is often considered to evolve through sexual selection favouring males with the most conspicuous plumage (Darwin, 1871; Owens & Hartley, 1998), but it could also be influenced by natural selection for more cryptic females because they are usually more exposed to predation during the nesting period (Wallace, 1889; Badyaev & Hill, 2003). Despite the commonness of avian sexual dichromatism, in many cases, humans cannot detect colour differences that are discernible to birds because of the differences in their visual systems. Birds can perceive both visible and ultraviolet (UV) wavelength radiation and they have a system of oil droplets associated with their photoreceptors that increases their colour discrimination efficiency (Vorobyev, 2003). As a consequence, avian coloration should be studied using objective techniques that are independent of human perception and allow more rigorous and biologically meaningful analyses (Bennett, Cuthill & Norris, 1994; Eaton, 2005). Spectral reflectance studies, and especially those that consider spectral sensitivity of avian photoreceptors, have revealed that a host of species previously considered to be sexually monochromatic actually possesses cryptic sexual dichromatism, having significant sexual differences in coloration that could include both the visible and the UV spectra (Eaton, 2005; Tubaro, Lijtmaer & Lougheed, 2005; Santos, Elwaed & Lumeij, 2006; Masello, Lubjuhn & Quillfeldt, 2009).

An interesting albeit vastly understudied phenomenon of bird's plumage is the occurrence of fluorescence that occurs when short wavelength radiation is absorbed by the feather and re-emitted at longer wavelengths. So far, it has been described mainly in Australian parrots, typically through the use of non-quantitative techniques such as illuminating museum study skins with black light (Völker, 1937; Boles, 1990; Hausmann *et al.*, 2003). Fluorescence is generally associated with the presence of pigments and, for the plumage of parrots, it has been attributed to a yellow pigment, possibly a psittacofulvin which molecular structure is still unknown, found in both yellow and green plumage patches (Völker,

1937; Boles, 1991; McGraw & Nogare, 2005). Plumage fluorescence has only been objectively described for the budgerigar *Melopsittacus undulatus*, an Australian psittacid species of the Platycercini tribe (Collar, 1997; Arnold, Owens & Marshall, 2002; Pearn, Bennett & Cuthill, 2003*a*) and no fluorescent sexual dimorphism was found in this species. Diverse and somehow contradictory results have been obtained when studying the role of fluorescence as a signal for mate attraction in the budgerigar, possibly because of differing methodological approaches (Pearn, Bennett & Cuthill, 2001; Arnold *et al.,* 2002; Pearn *et al*., 2003*a*; Pearn, Bennett & Cuthill, 2003*b*).

The blue-winged parrotlet *Forpus xanthopterygius* is a small member of the monophyletic Neotropical tribe of parrots (Arini), distributed mainly in central and eastern South America (Collar, 1997; Juniper & Parr, 1998). Blue-winged parrotlets possess non-cryptic sexual dichromatism (i.e. evident to the human eye) as the wing coverts, lower back and rump of males are blue, while these patches are green in females. The rest of their plumage is green, grading into yellower green in some areas. This species is divided into five or six subspecies depending on the source, and these vary slightly in plumage coloration and body size (Collar, 1997; Juniper & Parr, 1998; Clements, 2007). Given that fluorescence has been documented mostly in Eastern Hemisphere parrots and through non-quantitative approaches, our first objective was to assess whether the blue-winged parrotlet exhibits plumage fluorescence. Upon finding that it does, we then looked for sexual dichromatism in fluorescence and determined whether cryptic coloration to the human eye contributes to the sexual dichromatism of this species. Our results confirm the presence of fluorescence in the plumage of the blue-winged parrotlet and provide an objective description of fluorescence in a Neotropical psittacid, including a novel record of fluorescence occurring in a blue plumage patch. Additionally, we report for the first time the presence of fluorescent sexual dichromatism in a bird species and show cryptic sexual dichromatism of plumage reflectance in this species.

Methods

Spectrofluorometry

We measured fluorescence on feathers from the rump and chest of three individuals of each sex extracted from study skins of the subspecies *F. x. vividus,* deposited at the Museo Argentino de Ciencias Naturales 'Bernardino Rivadavia'. We only included samples of this subspecies to reduce the possibility of confounding subtle subspecific differences in colour (Juniper & Parr, 1998) with those between sexes. We mounted 12–15 feathers of each specimen on matt black paper (Quesada & Senar, 2006). We chose these two plumage patches to perform the measurements because the rump has non-cryptic sexual dichromatism but the chest appears sexually monochromatic to the human eye, and both plumage patches are large enough to allow us to take the necessary number of feathers with minimum possible damage to the study skins. Even though this sample size does not permit statistical comparisons, we restricted the spectrofluorometric analysis to three specimens for each sex to minimize destructive specimen sampling. We consider that this sample size is sufficient for a descriptive analysis of plumage fluorescence.

We measured fluorescence with a steady-state spectrofluorometer (QuantaMaster, PTI-Photon Technology International, Brunswick, NJ, USA) in front face geometry (the plumage was oriented 30° relative to the incident beam). This equipment illuminates the surface of the feathers with a monochromatic beam of light of a fixed wavelength (the excitation beam) that excites the fluorescence emission in the feathers. The emission of fluorescence is then recorded, for each different excitation wavelength used, as counts per second (cps) and as a function of wavelength within a given range of emission wavelengths. We recorded emission spectra varying the excitation wavelength between 300 and 700 nm, in increasing intervals of 10 nm, using a slit width of 3 nm for the excitation beam. The interval of emission wavelengths was selected so that their values started from 20 nm longer than the excitation wavelength and ended at 40 nm shorter than double this value. In this way, artefacts as a result of Rayleigh scattering (occurring when emission wavelength equals excitation wavelength) and second-order rays of Rayleigh scattering (taking place when the emission wavelength is twice as long as the excitation wavelength) were avoided. Emission spectra were recorded for increments of 1 nm within this range and they were additionally corrected by the detector response to each wavelength. We calculated median emission values for 5 nm bins to reduce the amount of data and to smooth the shape of the spectra. Finally, we averaged the emission spectra obtained for each patch and sex.

Following the same methodology described earlier, we measured fluorescence emission excited by UV light on positive and negative feather fluorescence references. As a positive reference, we used mounted feathers of the budgerigar's crown (this is the only bird species were plumage fluorescence was objectively measured and the crown is one of the plumage patches with the most intense fluorescence emission; Arnold *et al*., 2002; Pearn *et al*., 2003*a*). As a negative reference, we looked for black matte feathers, which present negligible light emission in the visible range, and selected the black tail feathers of the paradise tanager *Tangara chilensis*.

To determine if the fluorescence values obtained for the blue-winged parrotlet were significant from a photophysical perspective, we calculated the fluorescence quantum yield (φ_f), which indicates the ratio of the numbers of photons emitted as fluorescence to the number of photons absorbed by the sample (Lakowicz, 1999). We estimated this parameter for both plumage patches of each sex by applying a procedure used previously for the determination of fluorescence quantum yields in flower petals (Iriel & Lagorio, 2010*a*,*b* and references therein). Five determinations were done for each sample, using the spectrofluorometer described earlier:

(1) *Emission spectra (J_f).* Samples were excited in the UV (at 350 nm for female's rump, female's chest and male's chests and at 360 nm for male's rump). The selected excitation wavelength (350 or 360 nm) corresponded to maxima in the absorption spectrum of plumage. The emission spectra were recorded from a wavelength 10 nm longer than the excitation

wavelength to 700 nm. The integrated area under this emission spectrum represents the number of photons emitted by the sample as fluorescence.

(2) *Integrated reflected light from the blank standard (J_0).* The excitation beam was fixed at either 350 or 360 nm, and a blank standard of barium sulfate was placed in the sample holder. The light emerging from this sample (detected as usual as for an emission spectrum) was recorded from 335 to 365 nm (for excitation at 350 nm) and from 345 to 375 nm (for excitation at 360 nm). The integrated area under this curve stands for the scattered light from the blank. In this measurement, a NG4 glass transmission filter (Schott AG, Mainz, Germany) was placed at the beam exit to attenuate light and to avoid damaging the detector.

(3) *Integrated reflected light from the samples (J).* The excitation beam was fixed at either 350 or 360 nm and the feathers were placed in the sample holder. The light was collected from 335 to 365 nm and from 345 to 375 nm, respectively (as for J_0). The area under the curve represents the integrated scattered light from the sample. The emerging beam was passed through the NG4 filter before reaching the detector as for J_0.

(4) *Integrated scattered light intensity from the samples (I).* The excitation light was fixed at 700 nm and a group of feathers displaying negligible absorption at 700 nm was placed at the sample holder. The emerging light was recorded as a function of wavelength between 685 and 715 nm with the NG4 filter before the detector. The area under the curve represents the scattered light intensity from the sample at wavelength of 700 nm.

(5) *Integrated scattered light intensity from the blank (I_0).* The excitation beam was fixed at 700 nm (as for I) and the light was recorded between 685 and 715 nm for the blank using the NG4 filter before the detector.

The whole set of signals was corrected by the detector response to the different wavelengths. The experimental spectra obtained when measuring J_0, J, I and I_0 were additionally divided by the NG4 filter transmittance to provide the correct values. The observed fluorescence quantum yields were then calculated as

$$\phi_f = \frac{J_f}{J_0\left(I/I_0\right) - J} \quad (1)$$

In equation (1), the factor I/I_0 was introduced to correct for the differences in scattering properties between the blank and the feathers.

Spectrophotometry

Reflectance data were collected from museum study skins deposited at the Museo Argentino de Ciencias Naturales 'Bernardino Rivadavia'. To avoid confounding effects as a result of subspecific colour variation, we only used samples of the subspecies *F. x. vividus.* We sampled eight adult males and seven adult females. We collected reflectance spectra from five plumage patches (head, back, belly, chest and rump).

Plumage reflectance was measured with an Ocean Optics 2000 spectrophotometer (Ocean Optics, Inc., Dunedin, FL, USA) with a PX-2 pulsed xenon light source (effective range emission from 220 to 750 nm) calibrated against a white standard of barium sulfate. Plumage was illuminated and reflected light collected with a probe located in a holder, which isolates the surface from ambient light, at 45° from the surface in a proximal-distal orientation to reduce specular reflectance (Andersson, 1996). The distance between the probe tip and the plumage was 15 mm. The spectrophotometer resolution was 0.32 nm and each spectrum was the average of three readings, with an integration time of 100 ms. No boxcar smoothing was performed. The spectrophotometer was recalibrated before measuring each specimen.

We only worked with the reflectance readings between 320 and 700 nm as this range includes the wavelengths to which birds are sensitive (Montgomerie, 2006). To smooth the shape of the reflectance spectra and to reduce the data to manageable amounts, we calculated the median value for bins of 5 nm (Montgomerie, 2006). We extracted four variables from the reflectance spectra to describe plumage coloration (Montgomerie, 2006). Hue was defined as the wavelength of maximum reflectance ($\lambda_{R_{\max}}$). Brightness was calculated as total reflectance over the entire range of wavelengths ($\Sigma R_{320–700}$). Chroma, an indicator of signal spectral purity, was estimated as the ratio between reflectance 50 nm around the hue and brightness ($R_{(\lambda_{R_{\max}} \pm 50\ \mathrm{nm})}/R_{320–700}$). Finally, we calculated UV chroma as the relative UV reflectance (320–400 nm) to overall reflectance ($R_{320–400}/R_{320–700}$).

There is some evidence that plumage colour can fade as a result of museum storage, but the intensity of the change over each spectral variable depends on the mechanism of colour production involved and the methods employed to prepare and preserve the museum study skins (Armenta, Dunn & Whittingham, 2008; Doucet & Hill, 2009). This should anyway not affect our results because we did not find significant correlations between the year of capture of the specimens and the spectral variables for either sex (Spearman's correlations, $P > 0.05$ in all cases) and because the age of the study skins we used did not differ significantly between sexes (Mann–Whitney's $U = 15$, $P = 0.152$).

Statistical analyses

Because of small sample size and departures from normality in a few contrasts, we performed Mann–Whitney *U*-tests to compare the reflectance variables between sexes of each measured plumage patch. Statistical tests were performed with SPSS 15.0 for Windows (SPSS Inc., Chicago, IL, USA). Values are means ± standard deviation.

Results

Plumage fluorescence

Fluorescence emission intensity peaked at 522 nm in the chest of both sexes, corresponding to visible green (Fig. 1). The fluorescence emission of the green rump of females also had its maximum at 522 nm, while it peaked in the violet visible

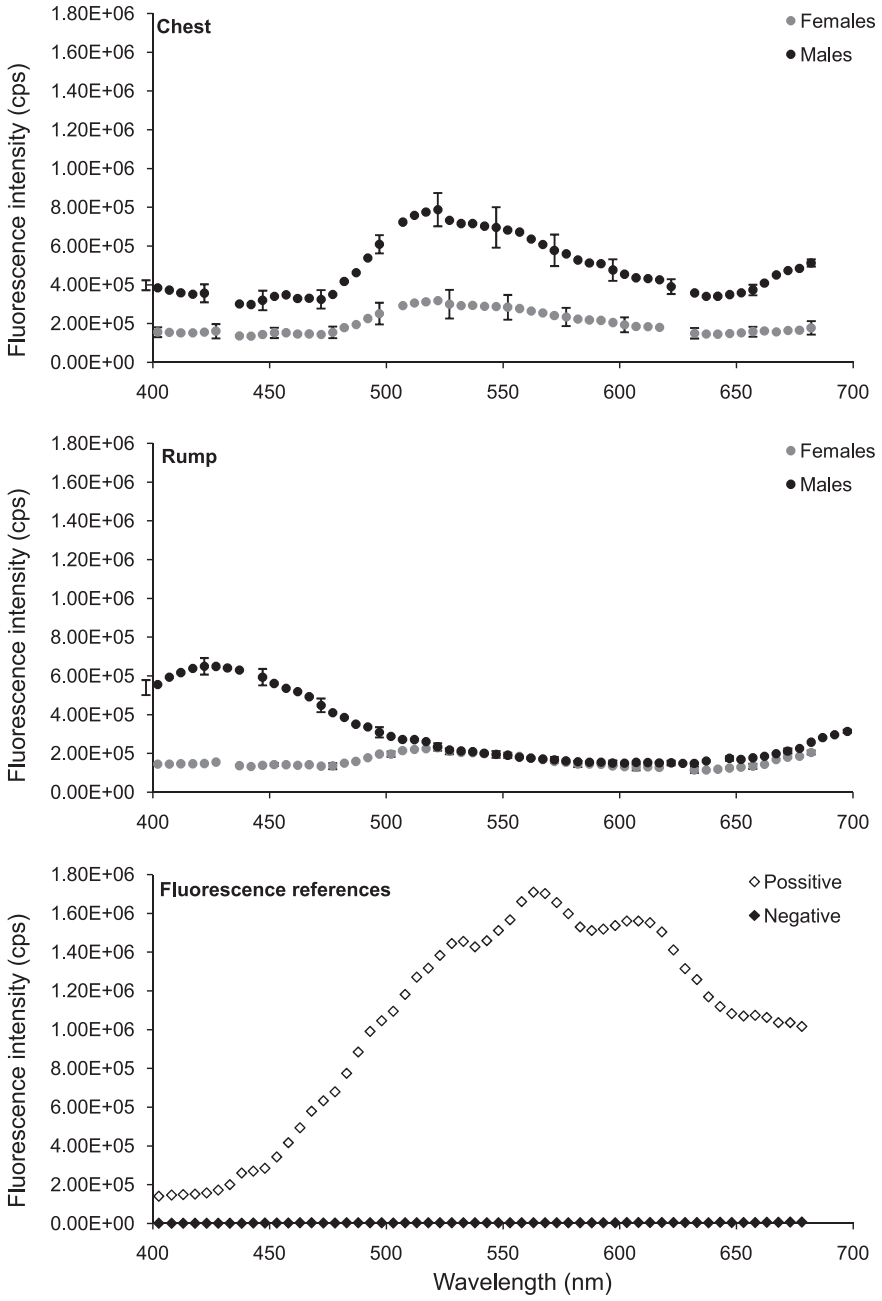

Figure 1 Mean fluorescence emission spectra obtained from rump and chest of males and females ($n = 3$ for both) of the blue-winged parrotlet *Forpus xanthopterygius* and for the positive and negative feather fluorescence references. Emission spectra represent mean fluorescence emission intensity ± SE obtained with the excitation wavelength that maximizes fluorescence emission for each plumage patch.

range (422 nm) for the blue rump of males (Fig. 1). Additionally, the shape of the fluorescence emission curve for this plumage patch differed between sexes (Fig. 1). Maximum emission was achieved when the excitation wavelength was set on 350 nm for the green plumage patches and with 360 nm for the rump of males. Maximum fluorescence emission for the males was at least twice that of females in both plumage patches. The highest values were obtained in the chest of males ($7.9 \times 10^5 \pm 1.8 \times 10^5$ cps), followed by the rump of males ($6.5 \times 10^5 \pm 0.8 \times 10^5$ cps). Female maximum fluorescence emission values were $3.2 \times 10^5 \pm 1.4 \times 10^5$ cps in the chest and $2.3 \times 10^5 \pm 0.3 \times 10^5$ cps in the rump. These values represent 14–46% of the maximum fluorescence intensity emitted by the feathers of the budgerigar's crown (positive feather fluorescence reference, 17×10^5 cps), while the maximum fluorescence emission recorded for the black feathers of the paradise tanager's tail used as negative fluorescence reference represent only 0.4% of the maximum emission of the positive reference (0.07×10^5 cps, Fig. 1).

The highest quantum yield value was that of rump of males with $\varphi_f = 0.042$ (i.e. 4.2% of absorbed photons are re-emitted as fluorescence), followed by their chest ($\varphi_f = 0.035$), then the

chest of females ($\varphi_f = 0.012$), and finally the rump of females with the lowest value ($\varphi_f = 0.010$).

Plumage reflectance

Reflectance spectra of both sexes and the five studied plumage patches are shown in Fig. 2, and the statistical analysis of sexual differences is presented in Table 1. In addition to the rump, coloured blue and green in males and females, respectively, we found significant differences of a few nanometres in the hue of the head with larger values in females resulting in a more yellowish green plumage. Females also exhibited higher hue values in the back, belly and chest, but these differences were not significant. Brightness, measured as total reflectance, was significantly higher in the rump of males, but no other plumage patch showed differences between sexes for this parameter. Chroma did not differ for any of the measured plumage areas. Finally, we found significant intersexual differences of UV chroma in the head, back, chest and rump of this species, with males having higher values than females for this parameter and therefore a higher UV reflectance in relation to total reflectance.

Discussion

Using quantitative and objective techniques of colour measurement, we showed that the plumage of the blue-winged parrotlet possesses UV-induced fluorescence and previously unrecognized sexual dichromatism involving both fluorescence and UV reflectance. We discuss these findings, in turn, below.

We found that maximum fluorescence emission occurred in the range of green wavelengths in the chest of both sexes and the rump of females, and in the violet range in the rump of males, coinciding in all cases with the observed hue of the feathers. Fluorescence production in parrots has been associated with the presence of yellow pigments, as it was registered in yellow and green plumage patches (Völker, 1937; Boles, 1991; McGraw & Nogare, 2005). Coincidentally, Hausmann *et al*. (2003) surveyed 51 parrot species of 24 different genera and found fluorescence emissions on 35 of them within the visible yellow, orange and green colours when illuminating museum study skins with a black light. We not only found fluorescence in green plumage patches of the blue-winged parrotlet, but also a violet fluorescence emission peak in the blue rump of males, which is suspected to have a purely structurally based coloration (R. O. Prum, pers. commun.). Pearn *et al*. (2003*a*) did not find significant fluorescence emission in the budgerigar's blue tail, but they found violet fluorescence emissions in the white downy feathers of male budgerigars. These results are surprising because the suspected structural origin of the coloration of these feathers would lead one to think that yellow pigments, and therefore fluorescence, would be absent.

Two plausible scenarios could explain the emission of fluorescence by the blue rump of blue-winged parrotlet males and the white downy feathers of the budgerigar. The alleged fluorescent yellow pigment could also be present in these feathers but its yellow coloration not perceived, either because it is in

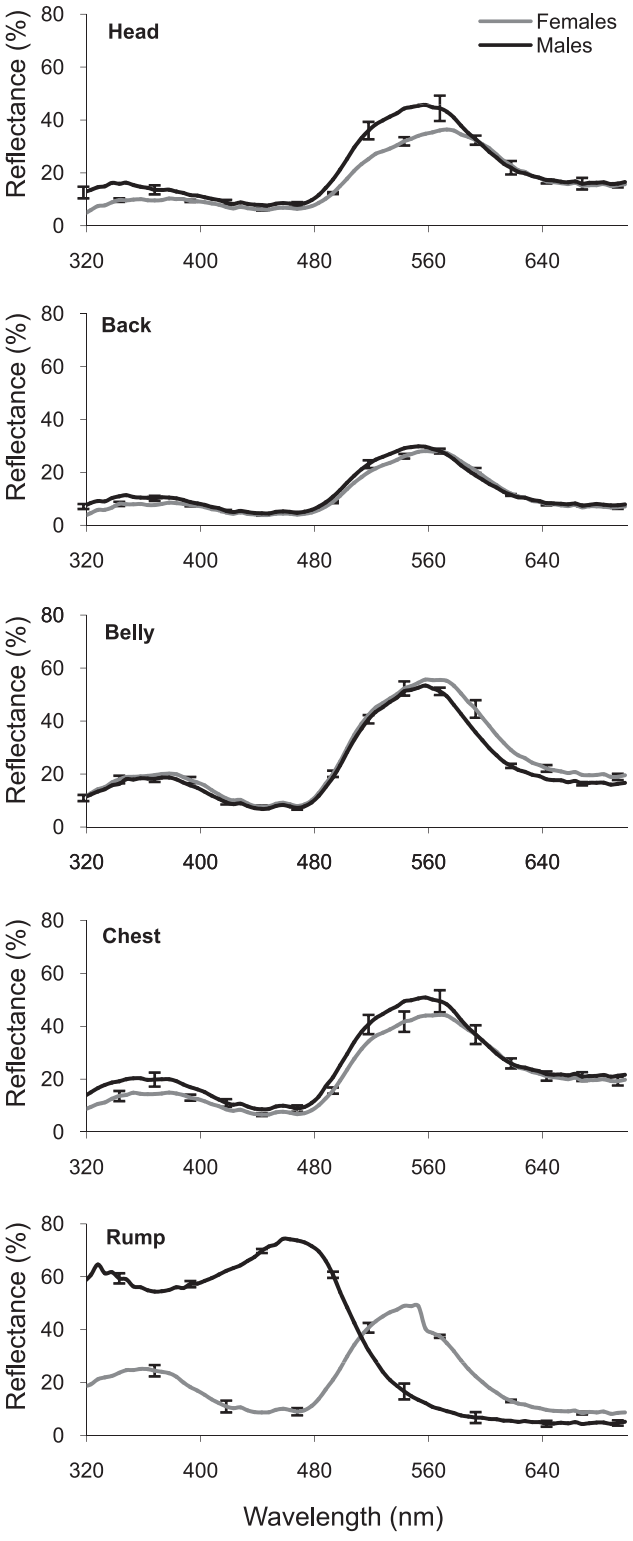

Figure 2 Mean reflectance spectra (±SE) for five plumage patches in male ($n = 8$) and female ($n = 7$) blue-winged parrotlets *Forpus xanthopterygius*.

Table 1 Sexual comparisons of plumage colour reflectance variables of the blue-winged parrotlet *Forpus xanthopterygius* for the five studied plumage patches

Plumage patch	Variable	Females ($n = 7$)	Males ($n = 8$)	U	P
Head	**Hue (nm)**	**573.49 ± 3.40**	**557.81 ± 8.01**	**1.0**	**0.001**
	Brightness	1291.80 ± 128.54	1591.70 ± 416.42	14.0	0.121
	Chroma	0.50 ± 0.03	0.51 ± 0.06	22.0	0.536
	UV chroma	**0.12 ± 0.01**	**0.15 ± 0.01**	**2.0**	**0.001**
Back	Hue (nm)	563.51 ± 6.74	554.70 ± 12.49	15.5	0.152
	Brightness	880.04 ± 84.18	973.62 ± 115.94	15.0	0.152
	Chroma	0.55 ± 0.04	0.52 ± 0.01	11.0	0.054
	UV chroma	**0.14 ± 0.02**	**0.17 ± 0.02**	**8.0**	**0.021**
Belly	Hue (nm)	564.94 ± 8.59	557.78 ± 2.65	15.5	0.152
	Brightness	1960.60 ± 280.27	1756.03 ± 158.04	17.0	0.232
	Chroma	0.50 ± 0.01	0.52 ± 0.02	17.0	0.232
	UV Chroma	0.15 ± 0.01	0.16 ± 0.02	23.0	0.613
Chest	Hue (nm)	564.26 ± 8.03	558.41 ± 8.18	18.5	0.281
	Brightness	1624.25 ± 379.95	1913.35 ± 495.83	15.0	0.152
	Chroma	0.49 ± 0.02	0.47 ± 0.03	18.0	0.281
	UV chroma	**0.13 ± 0.02**	**0.16 ± 0.02**	**6.0**	**0.009**
Rump	**Hue (nm)**	**549.97 ± 6.95**	**464.71 ± 9.24**	**19.9**	**0.000**
	Brightness	**1374.88 ± 302.59**	**2789.96 ± 172.29**	**0.0**	**0.000**
	Chroma	0.50 ± 0.03	0.48 ± 0.02	22.0	0.536
	UV chroma	**0.22 ± 0.02**	**0.35 ± 0.02**	**2.0**	**0.001**

Values are means ± standard deviation and Mann–Whitney U-test results for four estimated colour parameters as dependent variables with sex as the independent variable. Significant results are in bold.

low concentrations or because the feather's nanostructure precludes the reflection of such wavelengths (Shawkey & Hill, 2005). The fluorescent emission of this pigment would interact with the internal feather nanostructure when exiting and, as a consequence, we would detect wavelengths similar to those reflected by the plumage. This is consonant with the view of Stradi, Pini & Celentano (2001), who proposed that brilliant colours in parrots might result from the interaction between their yellow pigments and the plumage keratin. An alternate explanation would involve the emission of fluorescence by other constituent elements of the feather itself. Future studies of the pigments and structure of these feathers are needed to distinguish between these two possibilities.

Fluorescence emissions were about twice more intense in males than in females in the two plumage patches measured (chest and rump). Additionally, the shape of the fluorescence emission curve and the wavelength of its maximum in the rump differed between sexes. Even though the sample size did not allow statistical comparisons of these results, sexual differences are large and consistent among the individuals measured. These results are, to our knowledge, the first report of fluorescence sexual dichromatism in birds. The biological implications of fluorescence remain to be studied, as fluorescence could potentially act as a signal per se (Arnold *et al.,* 2002), it could function mainly by increasing the contrast between adjacent patches (Pearn *et al.,* 2001, 2003*b*; Hausmann *et al.,* 2003), or it could just be a by-product of the presence of unusual pigments in the plumage of parrots (Pearn *et al.,* 2003*a*).

Fluorescence quantum yield values estimated for the different patches provide a quantitative approximation of the photophysical relevance of the signal. The values of φ_f obtained indicate that the element that produces fluorescence in the rump plumage of males is more efficient in re-emitting the absorbed photons as fluorescence than that in the plumage of the chest of either sex and the rump of females. Our results indicate that the fluorescence found in the plumage of the blue-winged parrotlet is significant from a photophysical perspective, but its biological implications need to be tested.

Apart from detecting sexual differences in fluorescence, our analysis showed that the coloration of the blue-winged parrotlet possesses a more striking sexual dichromatism than previously recognized as a result of differences between the sexes in UV plumage reflectance. In fact, males had higher UV reflectance not only in the rump, which could be expected based on differences in the visible light spectrum, but also in the head, back and chest. Traditional descriptions of parrots consider sexual dichromatism to be limited to some genera (Collar, 1997), but cryptic differences such as these and those found in the budgerigar (Pearn *et al.,* 2003*a*) might actually be more common in the family than previously assumed (Santos *et al.,* 2006; Masello *et al.,* 2009). Human eyes are not sensitive to UV light and are less efficient to detect colour differences than avian ones and, therefore, these differences are not apparent without detailed spectrophotometric analysis; however, these could be large from an avian visual perspective (Eaton, 2005). There are no available data on the spectral sensitivity of the retinal cones of blue-winged parrotlets, but in the budgerigar (Bowmaker *et al.,* 1997), these are highly sensitive in the range of wavelengths where we found the main differences between the sexes.

UV coloration has been identified as an important signal for mate attraction in several bird species (Bennett *et al.,* 1997; Andersson, Örnborg & Andersson, 1998; Johnsen *et al.,* 1998), including the budgerigar and other parrot species (Pearn *et al.,* 2001; Hausmann *et al.,* 2003). Our findings imply that this also could be the case for the blue-winged parrotlet. Sexual displays by males in the genus *Forpus* include the exposure of their rumps to prospective females (Waltman & Beissinger, 1992), and this is the patch where we found the most intense sexual dichromatism and the highest proportion of UV reflectance. It would be interesting to explore the role that UV coloration might play, not only in a sexual context but also in other social interactions in blue-winged parrotlets.

In summary, we show the presence of UV-induced fluorescence and novel sexual differences in plumage coloration in the blue-winged parrotlet. We found fluorescence not only in green plumage patches of this species, which have been previously found to fluoresce in other species of parrots, but also report for the first time the presence of fluorescence in a blue plumage patch. Additionally, we document sexual dichromatism in fluorescence for the first time in a bird species and show that this species possesses a more striking sexual dichromatism than previously recognized as a result of the differences in UV reflectance in plumage patches that appear monochromatic to the human eye. Finally, to assess how general these findings are, we suggest that future analyses examine other psittacids, particularly other subspecies of blue-winged parrotlets and other members of the genus *Forpus* that possess similar patterns of plumage coloration.

Acknowledgements

We thank Richard O. Prum for his valuable comments regarding the mechanisms of fluorescence and reflectance production in the plumage of the blue-winged parrotlet. We also thank Walter E. Boles for providing us with a copy of his paper on fluorescence, and the anonymous reviewers for their valuable comments on a previous version of the paper. This work was supported by the Consejo Nacional de Investigaciones Científicas y Tecnológicas of Argentina (PIPs 5074 and 112-200801-00741, P.L.T.), a Natural Sciences and Engineering Research Council of Canada Discovery Grant and a Canada Foundation for Innovation New Opportunities Grant (S.C.L.), the University of Buenos Aires (Project UBACyT X114 2008–2010), the University of San Martín (Project SA08/009) and the Agencia Nacional de Promoción Científica y Tecnológica (BID 1201/OC-AR PICTs 11685 and 938, M.G.L.).

References

Andersson, S. (1996). Bright ultraviolet colouration in the Asian whistling-thrushes (*Myophonus* spp.). *Proc. Roy. Soc. Lond. Ser. B.* **263**, 843–848.

Andersson, S., Örnborg, J. & Andersson, M. (1998). Ultraviolet sexual dimorphism and assortative mating in blue tits. *Proc. Roy. Soc. Lond. Ser. B.* **265**, 445–450.

Armenta, J.K., Dunn, P.O. & Whittingham, L.A. (2008). Effects of specimen age on plumage color. *Auk* **125**, 803–808.

Arnold, K.E., Owens, I.P.F. & Marshall, N.J. (2002). Fluorescent signaling in parrots. *Science* **295**, 92.

Badyaev, A.V. & Hill, G.E. (2003). Avian sexual dichromatism in relation to phylogeny and ecology. *Annu. Rev. Ecol. Evol. Syst.* **34**, 27–49.

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., Partidge, J.C. & Lunau, K. (1997). Ultraviolet plumage colors predict mate preferences in starlings. *Proc. Natl. Acad. Sci. USA* **94**, 8618–8621.

Boles, W.E. (1990). Glowing parrots: need for a study of hidden colors. *Birds Int.* **3**, 76–79.

Boles, W.E. (1991). Black light signature for birds? *Aust. Nat. Hist.* **23**, 752.

Bowmaker, J.K., Heath, L.A., Wilkie, S.E. & Hunt, D.M. (1997). Visual pigments and oil droplets from six classes of photoreceptor in the retinas of birds. *Vision Res.* **37**, 2183–2194.

Clements, J.F. (2007). *The Clements checklist of the birds of the world*, 6th edn. Ithaca: Cornell University Press.

Collar, N.J. (1997). Family Pisttacidae (parrots). In *Handbook of the birds of the world, Vol. 4: Sandgrouse to Cuckoos*: 280–477. del Hoyo, J., Elliott, A. & Sargatal, A. (Eds). Barcelona: Lynx Edicions.

Darwin, C.R. (1871). *El origen del hombre y la selección en relación al sexo*. Madrid: EDAF.

Doucet, S.M. & Hill, G.E. (2009). Do museum specimens accurately represent wild birds? A case study of carotenoid, melanin, and structural colours in long tailed manakins *Chiroxiphia linearis*. *J. Avian. Biol.* **40**, 146–156.

Eaton, M.D. (2005). Human vision fails to distinguish widespread sexual dichromatism among sexually 'monochromatic' birds. *Proc. Natl. Acad. Sci. USA* **102**, 10942–10946.

Hausmann, F., Arnold, K.A., Marshall, N.J. & Owens, I.P.F. (2003). Ultraviolet signals in birds are special. *Proc. Roy. Soc. Lond. Ser. B.* **270**, 61–67.

Iriel, A. & Lagorio, M.G. (2010a). Implications of reflectance and fluorescence of *Rhododendron indicum* flowers in biosignaling. *Photochem. Photobiol. Sci.* **9**, 342–348.

Iriel, A. & Lagorio, M.G. (2010b). Is the flower fluorescence relevant in biocommunication? *Naturwissenschaften* **97**, 915–924.

Johnsen, A., Andersson, S., Örnborg, J. & Lifjeld, J.T. (1998). Ultraviolet plumage ornamentation affects social mate choice and sperm competition in bluethroats (Aves: Luscinia s. svecica): a field experiment. *Proc. Roy. Soc. Lond. Ser. B.* **265**, 1313–1318.

Juniper, T. & Parr, M. (1998). *Parrots: a guide to parrots of the world*. New Heaven: Yale University Press.

Lakowicz, J.R. (1999). *Principles of fluorescence spectroscopy*. 2nd edn. New York, London, Moscow, Dordrecht: Kluwer Academic/Plenum Publishers.

Masello, J.F., Lubjuhn, T. & Quillfeldt, P. (2009). Hidden dichromatism in the burrowing parrot (*Cyanoliseus patagonus*) as revealed by spectrometric colour analysis. *Hornero* **24**, 47–55.

McGraw, K.J. & Nogare, M.C. (2005). Distribution of unique red feather pigments in parrots. *Biol. Lett.* **1**, 38–43.

Montgomerie, R. (2006). Analyzing colors. In *Bird coloration Vol. I: mechanisms and measurements*: 90–147. Hill, G.E. & McGraw, K.J. (Eds). Cambridge: Harvard University Press.

Owens, I.P.F. & Hartley, I.R. (1998). Sexual dimorphism in birds: why are there so many different forms of dimorphism? *Proc. Roy. Soc. Lond. Ser. B* **265**, 397–407.

Pearn, S.M., Bennett, A.T.D. & Cuthill, I.C. (2001). Ultraviolet vision, fluorescence and mate choice in a parrot, the budgerigar *Melopsittacus undulatus*. *Proc. Roy. Soc. Lond. Ser. B* **268**, 2273–2279.

Pearn, S.M., Bennett, A.T.D. & Cuthill, I.C. (2003a). The role of ultraviolet-A reflectance and ultraviolet-A induced fluorescence in the appearance of budgerigar plumage: insights from spectrofluorometry and reflectance spectrophotometry. *Proc. Roy. Soc. Lond. Ser. B.* **270**, 859–865.

Pearn, S.M., Bennett, A.T.D. & Cuthill, I.C. (2003b). The role of ultraviolet-A-induced fluorescence in budgerigar mate choice. *Ethology* **109**, 961–970.

Quesada, J. & Senar, J.C. (2006). Comparing plumage colour measurements obtained directly from live birds and from collected feathers: the case of the great tit *Parus major*. *J. Avian Biol.* **37**, 609–616.

Santos, S.I., Elwaed, C.O.B. & Lumeij, J.T. (2006). Sexual dichromatism in the blue-fronted Amazon parrot (*Amazona aestiva*) revealed by multiple-angle spectrometry. *J. Avian Med. Surg.* **20**, 8–14.

Shawkey, M.D. & Hill, G.E. (2005). Carotenoids need structural colours to shine. *Biol. Lett.* **1**, 121–124.

Stradi, R., Pini, E. & Celentano, G. (2001). The chemical structure of the pigments in *Ara macao* plumage. *Comp. Biochem. Physiol. B Biochem. Mol. Biol.* **130**, 57–63.

Tubaro, P.L., Lijtmaer, D.A. & Lougheed, S.C. (2005). Cryptic dichromatism and seasonal color variation in the diademed tanager. *Condor* **107**, 648–656.

Völker, O. (1937). Ueber fluoreszierende, gelbe Federpigmente bei Papageien, eine neue Klasse von Federfarbstoffen. *J. Ornithol.* **85**, 136–146.

Vorobyev, M. (2003). Coloured oil droplets enhance colour discrimination. *Proc. Roy. Soc. Lond. Ser. B.* **270**, 1255–1261.

Wallace, A.R. (1889). *Darwinism: an exposition of the theory of natural selection with some of its applications*. London, New York: Macmillan and Co.

Waltman, J.R. & Beissinger, S.R. (1992). Breeding behavior of the green-rumped parrotlet. *Wilson Bull.* **104**, 65–84.