JOURNAL OF Evolutionary Biology

doi: 10.1111/j.1420-9101.2010.02166.x

The contribution of structural-, psittacofulvin- and melanin-based colouration to sexual dichromatism in Australasian parrots

A. J. TAYSOM*, D. STUART-FOX* & G. C. CARDOSO*†

*Department of Zoology, University of Melbourne, Melbourne, Vic., Australia
†CIBIO – Centro de Investigação em Biodiversidade e Recursos Genéticos, Campus Agrário de Vairão, Vairão, Portugal

Keywords:

colour ornamentation;
pigment colours;
sexual dichromatism;
sexual selection;
structural colour.

Abstract

Colour ornamentation in animals is exceptionally diverse, but some colours may provide better signals of individual quality or more efficient visual stimuli and, thus, be more often used as sexual signals. This may depend on physiological costs, which depend on the mechanism of colour production (e.g. exogenously acquired colouration in passerine birds appears to be most sexually dichromatic). We studied sexual dichromatism in a sample of 27 Australasian parrot species with pigment- (melanin and psittacofulvin) and structural-based colouration, to test whether some of these types of colouration are more prominent in sexual ornamentation. Unlike passerines, in which long wavelength colouration (yellow to red) usually involves exogenous and costly carotenoid pigments, yellow to red colouration in parrots is based on endogenously synthesized psittacofulvin pigments. This allows us to assess whether costly exogenous pigments are necessary for these plumage colours to have a prominent role in sexual signalling. Structural blue colouration showed the largest and most consistent sexual dichromatism, both in area and perceptually relevant chromatic differences, indicating that it is often ornamental in parrots. By contrast, we found little evidence for consistent sexual dichromatism in melanin-based colouration. Unlike passerines, yellow to red colouration was not strongly sexually dichromatic: although the area of colouration was generally larger in males, colour differences between the sexes were on average imperceptible to parrots. This is consistent with the idea that the prominent yellow to red sexual dichromatism in passerines is related to the use of carotenoid pigments, rather than resulting from sensory bias for these colours.

Introduction

Sexual ornamentation varies extensively among species, both in elaboration and in the type of traits employed as ornaments. However, certain traits may be more likely to be used as ornaments because of intrinsic properties that render them more efficient signals, for example, more attractive to intended receivers. There are two primary hypotheses to explain why some types of sexual signal may be favoured over others. First, the 'handicap hypothesis' suggests that some signals may be more reliable indicators of individual quality (e.g. condition), because they are costly to produce or maintain (Andersson, 1994; Zahavi & Zahavi, 1997). Second, the 'signal efficacy' hypothesis suggests that certain signals are better at stimulating receivers, either because they are more conspicuous within a given habitat or because they resemble an attractive object such as a food item (sensory drive; Endler, 1992). Attraction of conspecifics to particular signals may result in those signals being subject to a higher intensity of sexual selection, irrespective of energetic or physiological costs. These hypotheses are commonly invoked to explain the evolution of

Correspondence: Gonçalo C. Cardoso, CIBIO – Centro de Investigação em Biodiversidade e Recursos Genéticos, Campus Agrário de Vairão, 4485-661 Vairão, Portugal.
Tel.: (+351) 922245813; fax: (+351) 252661780;
e-mail: gcardoso@mail.icav.up.pt

conspicuous animal colouration. Some colour traits, however, could evolve because of either of these two mechanisms, yet in such cases, little attention has been paid to the relative importance of each.

Most animal colouration results from (i) the deposition of pigments acquired from the diet (e.g. carotenoids); (ii) of pigments synthesized from nonpigmentary precursors (e.g. melanins) and (iii) structural colouration arising from differential light reflection in microstructures of the tegument (Fox, 1976; Fitzpatrick, 1998). Whether these types of colouration differ in their ability to signal condition and whether some are more likely to be used as sexual signals (i.e. to be sexually selected) have been the subject of intense and ongoing debate (e.g. Jawor & Breitwisch, 2003; Smith *et al.*, 2006; Fitze *et al.*, 2007; Isaksson *et al.*, 2007; Vinkler & Albrecht, 2010). Traditionally, carotenoid-based colouration was thought to provide more reliable signals of condition than other types of colouration because of (i) the dependence of carotenoid colouration on food intake (e.g. Brush & Power, 1976, Slagsvold & Lifjeld 1985, Hill *et al.*, 2002) and (ii) trade-offs between the expression of carotenoid colouration and immune or antioxidant functions (Folstad & Karter, 1992; McGraw, 2005; but see also Smith *et al.*, 2006; Fitze *et al.*, 2007). This view is supported by substantial empirical evidence that carotenoid colouration indicates individual condition (e.g. Merilä *et al.*, 1999; Lindström & Lundström, 2001; Saks *et al.*, 2003 Horak *et al.*, 2004; Baeta *et al.*, 2008; Peters *et al.*, 2008; Mougeot *et al.*, 2010), sometimes in species where melanin colouration does not (McGraw & Hill, 2000; Rosen & Tarvin, 2006). Comparative studies have also shown that sexual dichromatism is more pronounced for carotenoid- than melanin-based colouration (North American Passerines: Gray, 1996; Cardueline finches: Badyaev & Hill, 2000). However, the more pronounced sexual dichromatism in carotenoid colours could also result from sensory biases for reds or yellows. Specifically, red and yellow colours could be particularly stimulating in species that include fruit in their diets because these colours indicate ripening, in turn having driven the evolution of visual sensitivity to these wavelengths (e.g. Regan *et al.*, 2001) and giving rise to sexual preferences. Similar scenarios are supported by studies showing that colour preferences originating in the foraging context can extend to the sexual context, and vice versa (Rodd *et al.*, 2002; Møller & Erritzøe, 2010). However, contrary to expectations of the sensory bias hypothesis, Gray (1996) found that sexual dichromatism in carotenoid colouration was larger in granivorous than frugivorous passerines. This is consistent with the 'handicap hypothesis' because carotenoid availability should more often be limiting in granivorous than frugivorous species, thus providing more reliable signals of condition.

In contrast to carotenoid colours, endogenously synthesized melanin pigments (black, brown, grey and chestnut hues) have generally been thought to play a less important role in sexual signalling (McGraw *et al.*, 2002; McGraw & Nogare, 2004). This is because of their apparently low production costs (but see McGraw 2008, for recent hypotheses on costs of melanin physiology), and because they serve a number of other functions such as protection from UV rays, thermoregulation and, in birds, feather strengthening and reducing abrasion (Bonser, 1995; McGraw, 2005). Structural colours (mostly blues, ultraviolet and iridescent hues) are understudied in terms of sexual signalling. However, recent evidence suggests that both melanin-based and structural-based colours can be honest signals of male quality (Jawor & Breitwisch, 2003; Siefferman & Hill, 2003, 2005a,b; Tarof *et al.*, 2005; Kemp, 2008a). Furthermore, it has been suggested that growing the regular and precise nanostructures required for consistent structural colour might indicate developmental stability or nutritional status (Fitzpatrick, 1998; McGraw *et al.*, 2002; Hill *et al.*, 2005; Siefferman & Hill, 2005a, 2007; Peters *et al.*, 2007; Kemp, 2008b). However, the likely condition dependence of structural colouration is debated (Prum, 2006), and several studies have found no significant correlation between blue structural colour and male reproductive success (Perrier *et al.*, 2002), female mate choice (Liu *et al.*, 2007) or indicators of individual quality such as parental care (Smiseth *et al.*, 2001; Balenger *et al.*, 2007). Consequently, the role of structural colouration in sexual signalling, relative to other types of colouration, remains unclear.

In this study, we adopted a comparative approach to test whether certain types of colouration are more sexually dichromatic than others, and thus more likely to be used as sexual signals. Specifically, we compared the extent of sexual dichromatism in (i) reds to yellows, (ii) black and (iii) UV-blues. We assume that the extent of sexual dichromatism provides an indicator of their role in sexual signalling, as opposed to other functions (e.g. thermoregulation or UV protection). We determined sexual dichromatism using spectroradiometric colour measurements and a model of animal colour perception (Vorobyev & Osorio, 1998; Vorobyev *et al.*, 1998), which enabled us to assess dichromatism that is perceptually relevant to our study animals. Our second aim was to evaluate more clearly whether yellow to red colour ornamentation is more likely explained by the 'handicap' or 'sensory bias' hypothesis. Thus, we chose to study a sample of parrot species (order Psittaciformes), in which bright yellow to red colouration is not produced by carotenoid pigments. Instead, bright yellow to red colouration in parrots is produced by a class of lipid-soluble, lipochrome pigments unique to them, psittacofulvins (Nemésio, 2001; Stradi *et al.*, 2001; McGraw & Nogare, 2004, 2005; McGraw, 2006; Berg & Bennett, 2010). In some species, patches of yellow are also accentuated by fluorescence (Nemésio, 2001; Hausmann *et al.*, 2002; Pearn *et al.*, 2003; Berg & Bennett, 2010), which is attributed to lipid-soluble pigments with a

physiology similar to the other psittacofulvins (McGraw & Nogare, 2005; Berg & Bennett, 2010). The reflectance spectra of psittacofulvin and carotenoid colouration are very similar, with a reflectance plateau at the long wavelengths (yellow to red) and a smaller peak in the ultraviolet region (e.g. McGraw & Nogare, 2005; Masello *et al.*, 2008; Toral *et al.*, 2008; Berg & Bennett, 2010). Unlike carotenoids, psittacofulvins are endogenously synthesized, and although they have anti-oxidant properties (Morelli *et al.*, 2003), they do not circulate in the bloodstream (McGraw & Nogare, 2004) and thus do not seem to be employed as physiological antioxidants. Knowledge of the physiology of psittacofulvins is still limited, but these differences (independence on exogenous pigments, and not being employed as circulating antioxidants) suggest that they are physiologically less costly than carotenoids. Consequently, the extent of sexual dichromatism in yellow to red colouration of parrots, compared to other ornamental colours, can give an indication of whether or not high physiological cost is necessary for red to yellow plumage colours to have a prominent role in sexual signalling.

Methods

We used bird skins at the ornithology collection from Museum Victoria to measure colour and the size of colour patches for three categories of colouration in male and female parrots: yellow to red psittacofulvin colouration, melanin black colouration and structural blue colouration. In some species, yellow patches are accentuated by fluorescence (absorbance of ultraviolet light and remittance at longer wavelengths; Nemésio, 2001; Berg & Bennett, 2010). When it exists, fluorescence modifies the reflectance spectra, and therefore, our reflectance measurements include it. As it was not our objective to study this relatively rare component of plumage colouration, we did not attempt to separate fluorescent effects from the remaining. We only studied structural blue colouration and did not include structural white colouration, because it is produced by a different mechanism (random rather than coherent scattering of light). We also did not study green colouration, which is very common in parrots, for two reasons. First, green has a wavelength intermediate between red and blue and, in parrots, is produced by a combination of psittacofulvin pigments plus blue structural colour (Nemésio, 2001; Berg & Bennett, 2010). Therefore, we cannot ascertain the extent to which each mechanism contributes to sexual dichromatism. Second, green can be cryptic colouration in parrots, rather than ornamental, because most parrots are commonly observed in tree canopies (Juniper & Parr, 1998), and therefore sexual dichromatism in green colouration may not be interpretable in terms of sexual selection.

We studied all taxa (species or subspecies well differentiated in colouration) in the ornithology collection at Museum Victoria that had all three of the colour categories mentioned earlier, and for which there existed in the collection a minimum of three specimens of each sex in good condition (e.g. not having bald patches, missing feathers or body parts). Except for very old or damaged specimens, plumage colour of museum skins is very similar to that of live birds (Armenta *et al.*, 2008; Doucet & Hill, 2009), so that museum-based data are useful to study plumage colouration. Most of the species excluded were the cockatoos, because the family Cacatuidae does not have blue structural colouration (Nemésio, 2001). We also excluded one parrot species (*Eclectus roratus*) that has reversed sexual dichromatism (Heinsohn *et al.*, 2005), which is exceptional in parrots. In total, we studied 27 taxa, most from Australia, but also species from Papua New Guinea and Indonesia.

For each taxon, we randomly chose up to five male and five female specimens, depending on availability (sample sizes in Fig. 1), subject to the constraint that the specimens were in good condition. We quantified three aspects of sexual dichromatism: differences between males and females in the relative size of colour patches, chromatic contrast between the sexes and achromatic (i.e. brightness) contrast between the sexes.

Sexual dimorphism in the size of colour patches

We photographed each specimen using a digital camera (Ixus 8515; Canon, Tokyo, Japan) set at 50 cm above the workbench on a tripod. Each specimen was photographed dorsally, laterally and ventrally, in the same posture, with the wings folded naturally across the sides of the body. For each specimen, we then estimated the relative area of the three plumage colour categories to account for differences in body size. First, we measured the areas for each type of colouration using image analyses tools (The GIMP v. 2.2, The GIMP Development Team) always confirming visually that the colour patches were selected correctly. We then converted these areas to percentage of body area in each photograph. Because the dorsal, ventral and lateral views each account for roughly 1/4th of the total body area, we calculated the total percentage body area for each type of colouration in each specimen as the weighted average of the photographs of the three body regions: $(1 \times \text{dorsal} + 1 \times \text{ventral} + 2 \times \text{lateral})/4$ and averaged this for the same-sex specimens of each taxon. For each taxon, we calculated the sexual dimorphism in area of colouration as the male minus the female relative patch sizes.

Chromatic and achromatic contrasts between the sexes

Spectroradiometry

Different parrot species have psittacofulvin- and structural-based colour patches in different parts of the body. For structural- or psittacofulvin-based colours, we

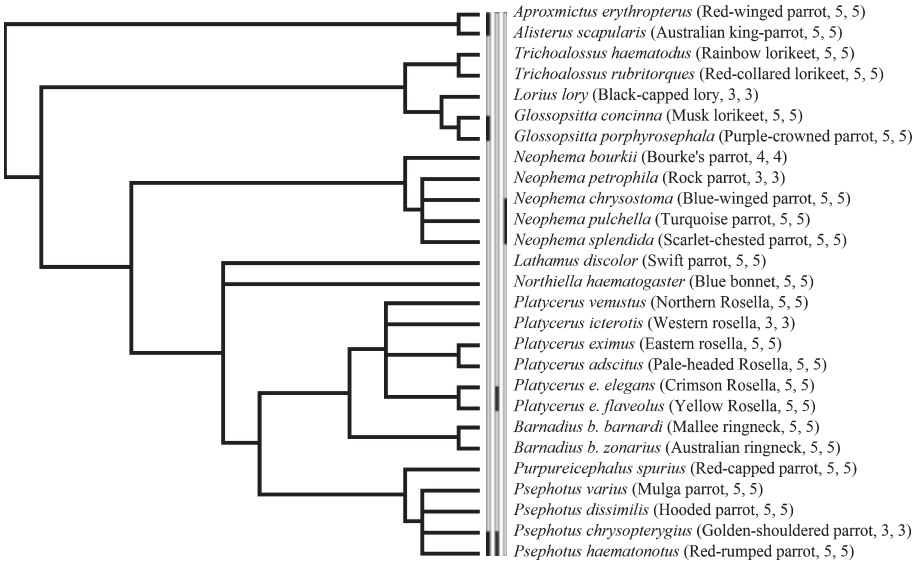

Fig. 1 Phylogeny comprising the taxa studied. Common names and number of male and female specimens measured are indicated in parenthesis. Dark regions in the three vertical bars mark the clades that were averaged to reduce the phylogenetic signal of, respectively, sexual dimorphism in the size of colouration patches, chromatic and achromatic contrast between the sexes (see text for details).

measured colour in the patch with the most conspicuous blue or red, respectively. We selected redder patches over orange and yellow, because red plumage requires higher concentrations of psittacofulvins compared to orange and yellow (McGraw & Nogare, 2005). If two or more patches of the same colour were present, the largest patch was selected. Melanin patches were consistently present on the wing primaries of the species studied, and thus melanin plumage was always measured from this area. Colour patches that were present in the same regions on both sexes were generally selected. This was not always possible because of differences in the location of colouration between the sexes. In such situations, the closest colour patch was measured instead.

We measured the reflectance of these colours from 300 to 700 nm, which encompasses the visible spectrum for birds (Burkhardt, 1989, Bowmaker *et al.*, 1999), with a USB 2000 + spectrophotometer, using a pulsed xenon light source (Ocean Optics Inc., Dunedin, FL, USA) and a R200 30°-angle UV fibre optic probe (fibre optics diameter of 200 μm, and 30°-angle of the probe tip). All data were generated relative to a diffuse reflectance white standard (Spectralon/PNAS-01158060). Spectrophotometric measurements in parrots are affected by the angle of measurement and light incidence, but repeatable using the same angle (Santos *et al.*, 2007). Therefore, we always used the same measurement angle (30° angled probe and light incidence, to avoid specular reflection) and always made measurements with the probe parallel to the direction of the rachis and facing the distal part of feathers.

For each type of colouration, we took five independent measurements per specimen and averaged the reflectance spectra. Then, we averaged these spectra across the different specimens of the same sex for each taxon. Finally, the reflectance spectra of each sex were smoothed by averaging reflectance over each interval of 5 nm, and these male and female spectra were used to calculate the chromatic and achromatic visual contrasts between sexes for each type of colouration.

Modelling visual contrasts

Birds, unlike humans, have tetrachromatic vision (Burkhardt, 1989; Bennett *et al.*, 1994; Hart & Vorobyev, 2005). That is, the retinae of parrots contain four classes of single cones, each containing a different visual pigment [longwave-sensitive (LWS), medium-wavelength-sensitive (MWS), short-wavelength-sensitive (SWS) and ultraviolet-sensitive (UVS)] associated with a particular type of oil droplet (Hart, 2001). Chromatic perception is a function of these four single cones (Kelber *et al.*, 2003), whereas achromatic vision and movement detection are thought to be attributed to double cones, which exist in much higher densities (Hart *et al.*, 1998). All birds studied to date fall into one of two highly conserved categories of visual systems, ultraviolet-sensitive (UVS) with a UVS cone peak sensitivity of around 360 nm and violet sensitive (VS) with a VS cone peak sensitivity of around 410 nm (Hart & Hunt, 2007). Parrots fall into the former category (Hart & Hunt, 2007); therefore, we used average photoreceptor spectral sensitivities for the UVS avian visual system, corrected for transmission of associated oil droplets, from Endler & Mielke (2005).

To analyse sexual dichromatism in plumage colour types based on the vision of parrots, we used the model

of Vorobyev & Osorio (1998), which is commonly applied to birds (e.g. Siddiqi *et al.*, 2004; Hastad *et al.*, 2005; Hemmi *et al.*, 2006; Stuart-Fox & Moussalli, 2008; McLean *et al.*, 2010). It assumes that visual discrimination is limited by photoreceptor noise, ω_i, and can be used to estimate the perceptual contrast between two colours (in this case male and female colour patches) in units of discrimination thresholds, or 'just noticeable differences' (JNDs). Model calculations are identical to those described in detail elsewhere (e.g. Stuart-Fox *et al.*, 2003; Stuart-Fox & Moussalli, 2008; McLean *et al.*, 2010).

Briefly, we first calculated receptor quantum catches (Q_i) for each cone type (i) for each colour patch:

$$Q_i = \int R_i(\lambda)S(\lambda)I(\lambda)\mathrm{d}\lambda \quad (1)$$

where $R_i(\lambda)$ is the spectral sensitivity of cone i, $S(\lambda)$ is the spectral reflectance of the colour patch, and $I(\lambda)$ is irradiance.

We used an irradiance measurement taken in full sun in woodland in the Southern Hemisphere Spring because this is the habitat occupied by most of the species studied. We used the average of three measures of downwelling irradiance taken using an SD2000 spectrometer and a calibrated, cosine-corrected irradiance probe (CC920 3-DA; Ocean Optics), under fine conditions, between 1000 and 1100 h. We then applied the von Kries transformation to account for receptor adaptation to the light environment, which contributes to colour constancy (Vorobyev & Osorio, 1998; Vorobyev *et al.*, 1998; Siddiqi *et al.*, 2004; Endler & Mielke, 2005):

$$q_i = k_i Q_i$$

Where

$$k_i = 1/\int R_i(\lambda)I(\lambda)\mathrm{d}\lambda \quad (2)$$

The receptor signal (f_i) is proportional to the natural logarithm of the quantum catch: $f_i = \log(q_i)$. The contrast for a tetrachromatic visual system can then be calculated using the following equation:

$$\begin{aligned}(\Delta S)^2 = {} & (\omega_1\omega_2)^2(\Delta f_4\Delta f_3)^2 + (\omega_1\omega_3)^2(\Delta f_4\Delta f_2)^2 \\ & + (\omega_1\omega_4)^2(\Delta f_3\Delta f_2)^2 + (\omega_2\omega_3)^2(\Delta f_4\Delta f_1)^2 \\ & + (\omega_2\omega_4)^2(\Delta f_3\Delta f_1)^2 + (\omega_3\omega_4)^2(\Delta f_2\Delta f_1)^2/((\omega_1\omega_2\omega_3)^2 \\ & + (\omega_1\omega_2\omega_4)^2 + (\omega_1\omega_3\omega_4)^2 + (\omega_2\omega_3\omega_4)^2) \end{aligned} \quad (3)$$

Where ΔS is the contrast between any two colours (in this case male and female colour patches) in units of JNDs, and ω_i is the noise-to-signal ratio (Weber fraction). Under bright illumination conditions, the Weber fraction can be calculated by:

$$\omega_i = v_i/\sqrt{\eta_i} \quad (4)$$

Where v_i is the noise-to-signal ratio of a single cone, and η_i is the number of photoreceptors of type i. We assumed that $\omega_i = 0.1$ for the LWS cone (Vorobyev *et al.*, 1998; Siddiqi *et al.*, 2004) and derived values of ω_i for the remaining photoreceptor classes from the equation above using a ratio of 1 UVS: 1.6 SWS: 3.3 MWS: 3.2 LWS for the four avian photoreceptor classes of an UVS visual system. This ratio is based on the mean ratio for five Psittacidae species from Hart (2001).

For calculations of chromatic or 'brightness' contrast, we used the equation f_D/ω_D where D is the double cone. We assumed that $\omega_i = 0.05$ for the double cone (Siddiqi *et al.*, 2004).

For the spectral sensitivity of the bird double cones, we used the sum of (i) the LWS sensitivity corrected for transmission of the oil droplet associated with the principal member of the double cone and (ii) the LWS sensitivity for the accessory member, which has no oil droplet. The resulting LWS spectral sensitivity was normalized to a maximum of 1 for calculations.

To check whether our results were sensitive to different irradiance measurements used in the models, we repeated all analyses using irradiance of woodland shade, but results were qualitatively the same, so we present only the results based on woodland sun.

Analyses

We derived three types of measure of sexual dichromatism (i) patch area dimorphism, (ii) visual chromatic contrast and (iii) visual achromatic contrast for each class of colouration (structural blue, psittacofulvin- and melanin-based colouration), giving a total of nine dichromatism variables. For each of the three types of measures of sexual dichromatism, we compared the degree of sexual dichromatism in the different classes of colouration using a repeated-measures ANOVA with the data grouped by taxon, followed by *post hoc* Tukey's tests.

This analysis assumes that sexual dichromatism in each taxon is at its adaptive optimum, not constrained by phylogenetic relations between species. This may be a reasonable assumption, because sexual dichromatism in birds is labile (Price & Birch, 1996; Omland, 1997; Hofmann *et al.*, 2008). However, to account for potential phylogenetic signal in the data, we repeated these analyses using an alternative approach.

Specifically, we reduced the phylogenetic signal of sexual dichromatism for all colour types in the dataset by selectively collapsing pairs of sister taxa, as follows. We first assembled a phylogeny for the studied species based primarily on Wright *et al.*'s (2008) mitochondrial DNA-based phylogeny. To this, we added one species, *Lathamus discolor*, based on the protein electrophoresis-based phylogeny of Christidis *et al.* (1991) and added congener species not included in the molecular phylogenies as polytomies (Fig. 1). We used branch lengths proportional to the number of taxa in each clade minus 1, as an approximation to the depth of nodes (Grafen, 1989; Garland *et al.*, 1992) that maintains extant species

equidistant from the root (Fig. 1). We calculated the phylogenetic signal, λ (Freckleton *et al.*, 2002), for each dichromatism variable using BayesTraits (M. Pagel and A. Meade, available from http://www.evolution.rdg.ac.uk). Then, we constructed multiple phylogenies, in each collapsing one of all the sister-taxa pairs into a single taxon, attributing to it the average phenotype of the collapsed taxa, and rearranging the branch lengths. In the case of polytomies, we collapsed each combination of pairs of taxa. We did this for every possible pair, each time recalculated λ and selected the phylogeny that yielded the lowest average λ of dichromatism for the three classes of coloration. We repeated this process until we obtained a phylogeny yielding an average phylogenetic signal lower than 0.1, indicating minimal phylogenetic signal. Clades that were collapsed for the final analyses are indicated in Fig. 1. We then conducted a repeated-measures ANOVA on the reduced set of taxa in this phylogeny as described earlier. In these analyses, similarities in dichromatism between different types of coloration should not be attributed to phylogenetic inertia because, within their range of variation, dichromatism was not constrained by phylogeny.

Results

Sexual dimorphism in the size of colour patches

Sex differences in the relative area of colour patches were significantly different among the three types of colouration ($F_{2,52} = 6.522$, $P = 0.006$). Sex differences in the size of melanin-coloured patches tended to be female-biased (male minus female difference in the relative percentage of area = −2.6% ± 2.1 SE, $N = 27$), contrary to the expected if these melanin patches were mostly ornamental. Psittacofulvin- and structural-based colour dichromatism was on average male-biased (i.e. males tended to have larger colour patches than females; male minus female difference for psittacofulvin colouration = 4.4% ± 1.2, and for structural colouration = 3.5% ± 1.1) and were significantly different from melanin-based sexual dichromatism (*post hoc* Tukey's tests: $q_{52,3} = 4.71$, $P < 0.01$, and $q_{52,3} = 4.07$, $P < 0.025$, respectively). Sex differences in the area of psittacofulvin-based plumage did not differ significantly from dichromatism in structural blue plumage ($q_{52,3} = 0.64$, $P > 0.5$).

Similarly, when using the reduced dataset with lowered phylogenetic signal, sex differences in the size of colour patches were significantly different among the three types of colouration ($F_{2,46} = 6.83$, $P = 0.005$, Fig. 2a). Psittacofulvin- and structural-based sexual dichromatism was again on average male-biased, and significantly different from melanin-based sexual dichromatism (Tukey tests: $q_{46,3} = 4.73$, $P < 0.01$, and $q_{46,3} = 4.29$, $P < 0.025$, respectively). Sex differences in the area of psittacofulvin-based plumage did not differ significantly from those in structural blue plumage ($q_{46,3} = 0.43$, $P > 0.5$).

Chromatic contrast

The chromatic contrast between the sexes was significantly different among the three types of colouration ($F_{2,52} = 17.05$, $P < 0.005$). Sexual dichromatism in structural blue (1.09 jnd ± 0.20) was significantly higher than dichromatism in psittacofulvin- and melanin-based colouration (0.46 jnd ± 0.09 and 0.05 ± 0.01, respectively; Tukey's tests: $q_{52,3} = 4.96$, $P < 0.005$, and $q_{52,3} = 8.20$, $P < 0.001$). Dichromatism in psittacofulvin- and melanin-based colouration was not significantly different ($q_{52,3} = 3.24$, $P > 0.05$) and was, on average lower than 1 jnd (the threshold below which the colours are indistinguishable), suggesting that chromatic differences between the sexes in those colours are generally not perceived by the birds. Even for structural blue

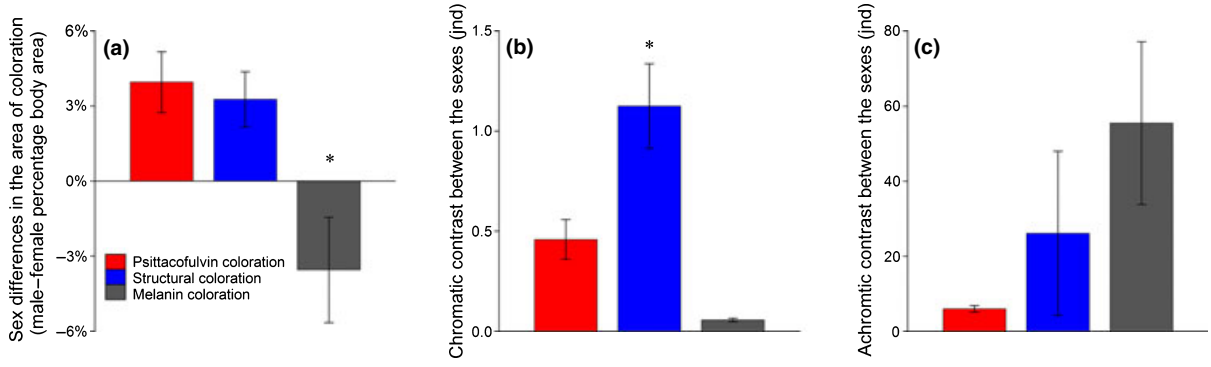

Fig. 2 Average (±SE) of (a) sexual dimorphism in the size of colouration patches, (b) chromatic and (c) achromatic contrast between the sexes for the three types of colouration studied, after reducing the phylogenetic signal of the data. Sample sizes in a = 24, b = 25 and c = 25. Asterisks indicate types of colouration whose sex difference differs significantly from the others in repeated-measures ANOVAS.

colouration, which had the highest chromatic contrast, the species average was only slightly above 1 jnd, suggesting that the chromatic differences are not biologically meaningful in all species.

Similarly, when using the dataset with reduced phylogenetic signal, the chromatic contrast between the sexes differed significantly between the types of colouration ($F_{2,48} = 16.12$, $P < 0.005$, Fig. 2b). Again, structural blue was the only plumage type with chromatic contrast on average above 1 jnd and was significantly higher than dichromatism in psittacofulvin- and melanin-based colouration (Tukey tests: $q_{48,3} = 4.96$, $P < 0.005$, and $q_{48,3} = 7.95$, $P < 0.001$). Dichromatism in psittacofulvin- and melanin-based colouration was again not significantly different ($q_{48,3} = 2.99$, $P > 0.05$).

Achromatic contrast

Using each taxon as an independent data point, the brightness or achromatic contrast between the sexes was marginally different among the three types of colouration ($F_{2,52} = 3.99$, $P = 0.05$), with psittacofulvin colouration being less sexually dichromatic than melanin-based colouration (5.73 jnd ± 0.82 and 84.3 ± 28.4, respectively; Tukey's test: $q_{52,3} = 3.83$, $P < 0.05$). The average value of sexual dichromatism in structural colouration was intermediate (24.6 jnd ± 20.2) between the other two types of colouration and not significantly different from either ($q_{52,3} = 2.9$, $P < 0.2$, and $q_{52,3} = 0.9$, $P > 0.5$, respectively). However, when using the reduced dataset with lowered phylogenetic signal, sexual dichromatism in brightness did not differ significantly between the three colour types ($F_{2,48} = 1.90$, $P = 0.32$, Fig. 2c). This suggests that the difference in brightness dichromatism found using each taxon as an independent point might have been augmented because of phylogenetic relatedness and must therefore be interpreted cautiously.

Discussion

We compared the degree of sexual dichromatism among the main types of pigment- and structural-based plumage colouration in a sample of Australasian parrot species. Sexual dimorphism in the area of coloured patches for psittacofulvin- and structural-based colours was male-biased, unlike melanin-based colouration. Sexual dichromatism evaluated as the chromatic contrast between the sexes was low for all colours, but the chromatic contrast for structural-based colouration was larger than for psittacofulvin- or melanin-based colouration. The latter two were on average lower than the discrimination threshold of the avian visual system. Because sexual dichromatism likely indicates the strength of sexual selection, this suggests that both psittacofulvin and structural colouration are often ornamental in parrots and that structural-based colouration may generally be more strongly sexually selected than psittacofulvin-based colouration.

We interpret the extent of sexual dichromatism as an index of the strength of sexual selection, because sexual selection is generally stronger on males (Shuster & Wade, 2003), and thus, sexual dimorphism in ornamentation tends to be male-biased (Andersson, 1994). Furthermore, several phylogenetic comparative studies have shown that sexual dichromatism covaries with the indices of sexual selection such as mating system, extra-pair copulation and sexual dimorphism in other traits (e.g. Badyaev, 1997; Owens & Hartley, 1998; Dunn *et al.*, 2001; Bennett & Owens, 2002), even in socially monogamous species (Møller & Birkhead, 1994). The most important limitation for using sexual dichromatism as an index of sexual selection is that it is influenced not only by the strength of sexual selection on males, but also by the extent to which sexual selection operates on females (Kraaijeveld *et al.*, 2007; Clutton-Brock, 2009) or genetic correlation between the sexes affecting the evolution of ornaments in females (Kraaijeveld & Reumer, 2008; Cardoso & Mota, 2010). Our results should be largely resilient to this shortcoming, because we restricted our analyses to comparisons of dichromatism within species, and the strength of sexual selection on males vs. females is held constant in within-species comparisons.

Our results showed that structural colouration was the type of ornamental colouration with the most consistent signs of sexual selection. Whether this pattern applies to birds more generally or is specific to parrots is currently unknown. When traits are consistently different across a clade – as was the case for dichromatism in structural colouration being larger than in other types of colouration – it is difficult to formally demonstrate that this is not because of an ancestral difference that was maintained in the clade. But this is unlikely to explain our results, because ornamental colouration and sexual dichromatism are highly evolutionarily labile in birds (Price & Birch, 1996; Omland, 1997; Hofmann *et al.*, 2008; Kraaijeveld & Reumer, 2008). Additionally, the little available empirical evidence on ornamental colouration in parrots (reviewed in Berg & Bennett, 2010) supports our finding that structural colours are likely to be sexually selected. A notable case study on burrowing parrots (*Cyanoliseus patagonus*) showed that the brightness of both psittacofulvin-based and structural colouration correlate with body condition or size and with conditions during rearing or moult (Masello *et al.*, 2004, 2008). There are different possible reasons for the prominent role of structural colouration in sexual signalling. Structural colours have been suggested to carry significant production and maintenance costs (Fitzpatrick, 1998; McGraw *et al.*, 2002; Hill *et al.*, 2005; Siefferman & Hill, 2005a, 2007; Peters *et al.*, 2007; Kemp, 2008b) and may also be favoured because they are potentially less conspicuous to predators (Hastad *et al.*, 2005).

We found no strong evidence for a widespread role of melanin-based colours in sexual signalling. In fact, sex differences in the size of melanin-coloured patches tended to be female-biased, instead of male-biased as would be typical of sexual ornaments. Furthermore, dichromatism in chromatic properties of melanin-based colouration was lower than that for structural colouration and, in terms of chromatic contrast, often not perceived by birds. The lack of strong sexual dichromatism in melanin-based colouration in parrots is consistent with the view that melanin is likely to be subjected to a range of selective pressures in both sexes, thereby limiting sexual dichromatism. For example, melanin colouration strengthens the feathers physically and to provides protection from abrasion (Bonser, 1995). This is especially useful at the wingtips, which are more susceptible to damage whilst airborne, and where all the species studied here had some black melanin colouration (Juniper & Parr, 1998).

Our results provide mixed evidence for the role of psittacofulvin-based colouration in sexual signalling. Sexual dimorphism in the size of yellow to red coloured patches was male-baised and similar to that for structural colours, suggesting that psittacofulvin-based colouration is ornamental in parrots. However, sex differences in the spectral properties of the pigmentation, evaluated as either chromatic or achromatic contrasts between the sexes, were not larger than any of the other types of colouration. On the contrary, the average chromatic contrast between the sexes for yellow to red colouration was less than for structural blue colouration and less than the birds' calculated threshold for colour discrimination, suggesting that these chromatic differences are not usually behaviourally relevant. The achromatic contrast between the sexes was perceptually larger for all colours, probably because the avian visual system is much more sensitive to gradations in brightness (Osorio & Vorobyev, 2005), but the contrast for red colouration still tended to be the smallest compared to the other types of colouration, and there was no significant difference between the three in the phylogenetically corrected analysis. Thus, overall, although the male-biased sexual dimorphism in patch size indicates that yellow to red colours are often ornamental, the other findings suggest that this is not a prominently sexually selected type of ornamentation, especially when compared to structural colouration.

Importantly, our results may be contrasted with sexual dichromatism in other avian groups, particularly passerines. Comparative studies in passerines show that the extent of dichromatism in carotenoid-based yellow to red colouration is greater than that in melanin-based colouration (Gray, 1996; Badyaev & Hill, 2000). In the two groups studied so far, Cardueline finches and North American passerines, yellow to red colouration seems to be the most sexually dimorphic type of colouration (Gray, 1996; Badyaev & Hill, 2000), although these studies did not assess sexual dichromatism in structural colouration. Our results point to a different scenario in parrots, in which yellow to red colouration is based on putatively less costly psittacofulvin pigments (McGraw & Nogare, 2004). Specifically, our results suggest that although yellow to red colours may have ornamental roles (as indicated by male-biased sexual dimorphism in patch size), dichromatism in psittacofulvin-based colouration was not greater than that for melanin colouration in terms of spectral properties and was generally less than the dichromatism in structural colouration. These results are similar to Kemp's (2008b) results for a butterfly, in which structural colouration is the most strongly sexually selected in comparison with endogenously synthesized, pteridin-based yellow colouration and melanin colouration. Together, our results support the idea that aspects of the physiology of carotenoids, and the costs they entail, contribute to the prominent role of yellow to red colouration in passerines (i.e. the 'handicap' hypothesis). Our finding that structural colouration is the most sexually dichromatic in parrots suggests that this may be a good taxonomic group to further investigate whether production and maintenance costs also contribute to the utility of structural colours in sexual signalling.

Acknowledgments

We thank Wayne Longmore for assistance with accessing the ornithological collection at Museum Victoria. This research was supported by a University of Melbourne Early Career Researcher Grant to G.C.C.

References

Andersson, M. 1994. *Sexual Selection*. Princeton University Press, Princeton, NJ.

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

Badyaev, A.V. 1997. Altitudinal variation in sexual dimorphism: a new pattern and alternative hypotheses. *Behav. Ecol.* **8**: 675–690.

Badyaev, A.V. & Hill, G.E. 2000. Evolution of sexual dichrornatism: contribution of carotenoid- versus melanin-based coloration. *Biol. J. Linn. Soc.* **69**: 153–172.

Baeta, R., Faivre, B., Motreuil, S., Gaillard, M. & Moreau, J. 2008. Carotenoid trade-off between parasitic resistance and sexual display: an experimental study in the blackbird (*Turdus merula*). *Proc. R. Soc. B* **275**: 427–434.

Balenger, S.L., Johnson, L.S., Brubaker, J.L. & Ostlind, E. 2007. Parental effort in relation to structural plumage coloration in the mountain bluebird (*Sialia currucoides*). *Ethology* **113**: 838–846.

Bennett, P.M. & Owens, I.P.F. 2002. *Evolutionary Ecology of Birds*. Oxford University Press, Oxford.

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

Berg, M.L. & Bennett, A.T.D. 2010. The evolution of plumage colouration in parrots: a review. *Emu* **110**: 10–20.

Bonser, R.H.C. 1995. Melanin and the abrasion resistance of feathers. *Condor* **97**: 590–591.
Bowmaker, J.K., Heath, L.A., Wilkie, S.E. & Hunt, D.M. 1999. Visual pigments and oil droplets from six classes of photoreceptor in the retinas of birds. *Vision Res.* **37**: 2183–2194.
Brush, A.H. & Power, D.M. 1976. House finch pigmentation: carotenoid metabolism and the effect of diet. *Auk* **93**: 725–739.
Burkhardt, D. 1989. UV vision: a bird's eye view of feathers. *J. Comp. Physiol. A.* **164**: 787–796.
Cardoso, G.C. & Mota, P.G. 2010. Evolution off emale coloration by sexual constraint in *Carduelis* finches. *BMC Evol. Biol.* **10**: 82.
Christidis, L., Schodde, R., Shaw, D.D. & Maynes, S.F. 1991. Relationships among the Australo-Papuan parrots, lorikeets and cockatoos (Aves: Psittaciformes): protein evidence. *Condor* **93**: 302–317.
Clutton-Brock, T. 2009. Sexual selection in females. *Anim. Behav.* **77**: 3–11.
Doucet, S.M. & Hill, G. 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.
Dunn, P.O., Whittingham, L.A. & Pitcher, T.E. 2001. Mating systems, sperm competition and the evolution of sexual dimorphism in birds. *Evolution* **55**: 161–175.
Endler, J.A. 1992. Signals, signal conditions, and the direction of evolution. *Am. Nat.* **139**: S125–S153.
Endler, J.A. & Mielke, P.W. 2005. Comparing entire colour patterns as birds see them. *Biol. J. Linn. Soc.* **86**: 405–431.
Fitze, P.S., Tschirren, B., Gasparini, J. & Richner, H. 2007. Carotenoid-based plumage colors and immune function: is there a trade-off for rare carotenoids? *Am. Nat.* **169**: S137–S144.
Fitzpatrick, S. 1998. Colour schemes for birds: structural colouration and signals of quality in feathers. *Ann. Zool. Fenn.* **35**: 67–77.
Folstad, I. & Karter, A.K. 1992. Parasites, bright males and the immunocompetence handicap. *Am. Nat.* **139**: 603–622.
Fox, D.L. 1976. *Animal Biochromes and Structural Colors*. University of California Press, Berkeley, CA.
Freckleton, R.P., Harvey, P.H. & Pagel, M. 2002. Phylogenetic analysis and comparative data: a test and review of evidence. *Am. Nat.* **160**: 712–726.
Garland, T. Jr, Harvey, P.H. & Ives, A.R. 1992. Procedures for the analysis of comparative data using phylo-genetically independent contrasts. *Syst. Biol.* **41**: 18–32.
Grafen, A. 1989. The phylogenetic regression. *Philos. Trans. Royal Soc. B* **326**: 119–157.
Gray, D.A. 1996. Carotenoids and sexual dichromatism in North American passerine birds. *Am. Nat.* **148**: 453–480.
Hart, N.S. 2001. Variations in cone photoreceptor abundance and the visual ecology of birds. *J. Comp. Physiol. A.* **187**: 685–698.
Hart, N.S. & Hunt, D.M. 2007. Avian visual pigments: characteristics, spectral tuning, and evolution. *Am. Nat.* **169**: S7–S26.
Hart, N.S. & Vorobyev, M. 2005. Modelling oil droplet absorption spectra and spectral sensitivities of bird cone photoreceptors. *J. Comp. Physiol. A.* **191**: 381–392.
Hart, N.S., Partridge, J.C. & Cuthill, I.C. 1998. Visual pigments, oil droplets and cone photoreceptor distribution in the European starling (*Sturnus vulgaris*). *J. Exp. Biol.* **201**: 1433–1446.
Hastad, O., Victorsson, J. & Odeen, A. 2005. Differences in color vision make passerines less conspicuous in the eyes of their predators. *Proc. Natl. Acad. Sci. USA* **102**: 6391–6394.
Hausmann, F., Arnold, K.E., Marshall, N.J. & Owens, I.P.F. 2002. Ultraviolet signals in birds are special. *Proc. R. Soc. B* **270**: 61–67.
Heinsohn, R., Legge, S. & Endler, J.A. 2005. Extreme reversed sexual dichromatism in a bird without sex role reversal. *Science* **309**: 617–619.
Hemmi, J.M., Marshall, J., Pix, W., Vorobyev, M. & Zeil, J. 2006. The variable colours of the fiddler crab Uca vomeris and their relation to background and predation. *J. Exp. Biol.* **209**: 4140–4153.
Hill, G.E., Inouye, C.Y. & Montgomerie, R. 2002. Dietary carotenoids predict plumage coloration in wild house finches. *Proc. R. Soc. B* **269**: 1119–1124.
Hill, G.E., Doucet, S.M. & Buchholz, R. 2005. The effect of coccidial infection on iridescent plumage coloration in wild turkeys. *Anim. Behav.* **69**: 387–394.
Hofmann, C.M., Cronin, T.W. & Omland, K.E. 2008. Evolution of sexual dichromatism carotenoids and melanins contribute to sexual dichromatism in new world orioles (*Icterus* spp.). *Auk* **125**: 790–795.
Horak, P., Saks, L., Karu, U., Ots, I., Surai, P.F. & McGraw, K.J. 2004. How coccidian parasites affect health and appearance of greenfinches. *J. Anim. Ecol.* **73**: 935–947.
Isaksson, C., Von Post, M. & Andersson, S. 2007. Sexual, seasonal, and environmental variation in plasma carotenoids in great tits, *Parus major*. *Biol. J. Linn. Soc.* **92**: 521–527.
Jawor, J.M. & Breitwisch, R. 2003. Melanin ornaments, honesty, and sexual selection. *Auk* **120**: 249–265.
Juniper, T. & Parr, M. 1998. *Parrots A. Guide to Parrots of the World*. Yale University Press, New Haven.
Kelber, A., Vorobyev, M. & Osorio, D. 2003. Animal colour vision: behavioural tests and physiological concepts. *Biol. Rev.* **78**: 81–118.
Kemp, D.J. 2008a. Female mating biases for bright ultraviolet iridescence in the butterfly *Eurema hecabe* (Pieridae). *Behav. Ecol.* **19**: 1–8.
Kemp, D.J. 2008b. Resource-mediated condition dependence in sexually dichromatic butterfly wing coloration. *Evolution* **62**: 2346–2358.
Kraaijeveld, K. & Reumer, B.M. 2008. Constraints and the evolution of mutual ornamentation. In: *Animal Behavior: New Research* (E.A. Weber & L.H. Krause, eds), pp. 193–213. Nova Science Publishers, New York.
Kraaijeveld, K., Kraaijeveld-Smit, F.J.L. & Komdeur, J. 2007. The evolution of mutual ornamentation. *Anim. Behav.* **74**: 657–677.
Lindström, K. & Lundström, J. 2001. Male greenfinches (*Carduelis chloris*) with brighter ornaments have higher virus infection clearance rate. *Behav. Ecol. Sociobiol.* **48**: 44–51.
Liu, M., Siefferman, L. & Hill, G.E. 2007. An experimental test of female choice relative to male structural coloration in eastern bluebirds. *Behav. Ecol. Sociobiol.* **61**: 623–630.
Masello, J.F., Pagnossin, M.L., Lubjuhn, T. & Quillfeldt, P. 2004. Ornamental non-carotenoid red feathers of wild burrowing parrots. *Ecol. Res.* **19**: 421–432.
Masello, J.F., Lubjuhn, T. & Quillfeldt, P. 2008. Is the structural and psittacofulvin-based colouration of wild burrowing parrots *Cyanoliseus patagonus* condition dependent? *J. Avian Biol.* **39**: 653–662.
McGraw, K.J. 2005. The antioxidant function of many animal pigments: are there consistent health benefits of sexually selected colourants? *Anim. Behav.* **69**: 757–764.

McGraw, K.J. 2006. Mechanics of uncommon colors: pterins, porphyrins, and psittacofulvins. In: *Bird Coloration, vol. I. Mechanisms and Measurements* (G.E. Hill & K. McGraw, eds), pp. 354–398. Harvard University Press, Cambridge, MS.

McGraw, K.J. 2008. An update on the honesty of melanin-based color signals in birds. *Pigment Cell Melanoma Res.* **21**: 133–138.

McGraw, K.J. & Hill, G.E. 2000. Differential effects of endoparasitism on the expression of carotenoid- and melanin-based ornamental coloration. *Proc. R. Soc. B* **267**: 1525–1531.

McGraw, K.J. & Nogare, M.C. 2004. Carotenoid pigments and the selectivity of psittacofulvin-based colouration systems in parrots. *Comp. Biochem. Physiol. B* **138**: 229–233.

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

McGraw, K.J., Mackillop, E.A., Dale, J. & Hauber, M.E. 2002. Different colors reveal different information: how nutritional stress affects the expression of melanin- and structurally based ornamental plumage. *J. Exp. Biol.* **205**: 3747–3755.

McLean, C.A., Moussalli, A. & Stuart-Fox, D. 2010. The predation cost of female resistance. *Behav. Ecol.* **21**: 861–867.

Merilä, J., Sheldon, B.C. & Lindström, K. 1999. Plumage brightness in relation to haemetozoan infections in the greenfinch *Carduelis chloris*: bright males are a good bet. *Ecoscience* **6**: 12–18.

Møller, A.P. & Birkhead, T.R. 1994. The evolution of plumage brightness in birds is related to extrapair paternity. *Evolution* **48**: 1089–1100.

Møller, A.P. & Erritzøe, J. 2010. Why birds eat colourful grit: colour preferences revealed by the colour of grizzard stones. *J. Evol. Biol.* **23**: 509–517.

Morelli, R., Loscalzo, R., Stradi, R., Bertelli, A. & Falchi, M. 2003. Evaluation of the antioxidant activity of new carotenoid-like compounds by electron paramagnetic resonance. *Drugs Exp. Clin. Res.* **29**: 95–100.

Mougeot, F., Martínez-Padilla, J., Bortolotti, G.R., Webster, L.M.I. & Piertney, S.B. 2010. Physiological stress links parasites to carotenoid-based colour signals. *J. Evol. Biol.* **23**: 643–650.

Nemésio, A. 2001. Colour production and evolution in parrots. *Internat. J. Ornithol.* **4**: 75–102.

Omland, K.E. 1997. Examining two standard assumptions of ancestral reconstructions: repeated loss of dichromatism in dabbling ducks (Anatini). *Evolution* **51**: 1636–1646.

Osorio, D. & Vorobyev, M. 2005. Photoreceptor spectral sensitivities in terrestrial animals: adaptations for luminance and colour vision. *Proc. R. Soc. B* **272**: 1745–1752.

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

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

Perrier, G., De Lope, F., Moller, A.P. & Ninni, P. 2002. Structural coloration and sexual selection in the barn swallow *Hirundo rustica*. *Behav. Ecol.* **13**: 728–736.

Peters, A., Delhey, K., Johnsen, A. & Kempenears, B. 2007. The condition-dependent development of carotenoid-based and structural plumage in nestling blue tits: males and females differ. *Am. Nat.* **169**: S122–S136.

Peters, A., Delhey, K., Andersson, S., Noordwijk, H.V. & Forschlert, M.I. 2008. Condition-dependence of multiple carotenoid-based plumage traits: an experimental study. *Funct. Ecol.* **22**: 831–839.

Price, T. & Birch, G.L. 1996. Repeated evolution of sexual colour dimorphism in passerine birds. *Auk* **113**: 842–848.

Prum, R.O. 2006. Anatomy, physics and evolution of avian structural colours. In: *Bird Coloration Vol. I: Mechanisms and Measurements* (G.E. Hill & K.J. McGraw, eds), pp. 295–353. Harvard Univ Press, Cambridge, MA.

Regan, B.C., Juliot, C., Simmen, B., Viénot, F., Charles-Dominique, P.C. & Mollon, J.D. 2001. Fruits, foliage and the evolution of primate colour vision. *Philos. Trans. R Soc. B* **356**: 229–283.

Rodd, F.H., Hughes, K.A., Grether, G.F. & Baril, C.T. 2002. A possible non-sexual origin of mate preference: are male guppies mimicking fruit? *Proc. R. Soc. B* **269**: 475–481.

Rosen, R.F. & Tarvin, K.A. 2006. Sexual signals of the male american goldfinch. *Ethology* **112**: 1008–1019.

Saks, L., Ots, I. & Horak, P. 2003. Carotenoid-based plumage coloration of male greenfinches reflects health and immunocompetence. *Oecology* **134**: 301–307.

Santos, S.I.C.O., De Neve, L., Lumeij, J.T. & Förschler, M.I. 2007. Strong effects of various incidence and observation angles on spectrometric assessment of plumage colouration in birds. *Behav. Ecol. Sociobiol.* **6**: 1499–1506.

Shuster, S.M. & Wade, M.J. 2003. *Mating Systems and Strategies*. Princeton University Press, Princeton.

Siddiqi, A., Cronin, T.W., Loew, E.R., Vorobyev, M. & Summers, K. 2004. Interspecific and intraspecific views of color signals in the strawberry poison frog Dendrobates pumilio. *J. Exp. Biol.* **207**: 2471–2485.

Siefferman, L. & Hill, G.E. 2003. Structural and melanin coloration indicate parental effort and reproductive success in male eastern bluebirds. *Behav. Ecol.* **14**: 855–861.

Siefferman, L. & Hill, G.E. 2005a. Evidence for sexual selection on structural plumage coloration in female eastern bluebirds (*Sialia sialis*). *Evolution* **59**: 1819–1828.

Siefferman, L. & Hill, G.E. 2005b. UV-blue structural coloration and competition for nest boxes in male eastern bluebirds. *Anim. Behav.* **69**: 67–72.

Siefferman, L. & Hill, G.E. 2007. The effect of rearing environments on blue structural coloration of eastern bluebirds (*Sialia sialis*). *Behav. Ecol. Sociobiol.* **61**: 1839–1846.

Slagsvold, T. & Lifjeld, J.T. 1985. Variation in plumage colour of the great tit *Parus major* in relation to habitat, season and food. *J. Zool.* **206**: 321–328.

Smiseth, P.T., Ornborg, J., Andersson, S. & Amundsen, T. 2001. Is male plumage reflectance correlated with paternal care in bluethroats? *Behav. Ecol.* **12**: 164–170.

Smith, H.G., Råberg, L., Ohlsson, T., Granbom, M. & Hasselquist, D. 2006. Carotenoid and protein supplementation have differential effects on pheasant ornamentation and immunity. *J. Evol. Biol.* **20**: 310–319.

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

Stuart-Fox, D. & Moussalli, A. 2008. Selection for social signalling drives the evolution of chameleon colour change. *PLoS Biol.* **6**: e25.

Stuart-Fox, D., Moussalli, A., Marshall, J. & Owens, I.P.F. 2003. Conspicuous males suffer higher predation risk: visual modelling and experimental evidence from lizards. *Anim. Behav.* **66**: 541–550.

Tarof, S.A., Dunn, P.O. & Whittingham, L.A. 2005. Dual functions of a melanin-based ornament in the common yellowthroat. *Proc. R. Soc. B* **272**: 1121–1127.

Toral, G.M., Figuerola, J. & Negro, J.J. 2008. Multiple ways to become red: pigment identification in red feathers using spectrometry. *Comp. Biochem. Physiol.* **150**: 147–152.

Vinkler, M. & Albrecht, T. 2010. Carotenoid maintenance handicap and the physiology of carotenoid-based signalisation of health. *Naturwissenschaften* **97**: 19–28.

Vorobyev, M. & Osorio, D. 1998. Receptor noise as a determinant of colour thresholds. *Proc. R. Soc. B* **265**: 351–358.

Vorobyev, M., Osorio, D., Bennett, A.T.D., Marshall, N.J. & Cuthill, I.C. 1998. Tetrachromacy, oil droplets and bird plumage colours. *J. Comp. Physiol. A.* **183**: 621–633.

Wright, T.F., Schirtzinger, E.E., Matsumoto, T., Eberhard, J.R., Graves, G.R., Sanchez, J.J., Capelli, S., Muller, H., Scharpegge, J., Chambers, G.K. & Fleischer, R.C. 2008. A multilocus molecular phylogeny of the parrots (Psittaciformes): support for a Gondwanan origin during the Cretaceous. *Mol. Biol. Evol.* **25**: 2141–2156.

Zahavi, A. & Zahavi, A. 1997. *The Handicap Principle*. Oxford University Press, New York.

Received 9 June 2009; revised 30 September 2010; accepted 1 October 2010