

Ultraviolet reflectance influences female preference for colourful males in the European serin

Ana V. Leitão · Anabela H. Monteiro · Paulo G. Mota

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Abstract Avian plumage colouration is one of the most impressive displays in nature and is frequently used as sexual signal. There is now considerable evidence that females consistently prefer males with the most elaborated colour displays. Bird colour vision expands into the ultraviolet (UV) range, which prompted several studies to test the importance of UV in mate choice, revealing that females are affected by the UV light component. These studies were mostly performed on structural plumage, whereas carotenoid-based plumage was rarely considered, although it also has a typical reflection peak in the UV. Our study tested the female choice over male yellow colouration, and whether it is influenced by UV removal, in the European serin (*Serinus serinus*), a sexually dichromatic cardueline finch, with males showing a conspicuous carotenoid-based yellow plumage. We shows that females preferred yellower males and that male attractiveness was lost when the UV colouration was blocked, with either of the UV-blocking techniques used. The results of our study indicate that the UV component of carotenoid colouration is important in the female mate assessment in serins and highlights the importance of considering colour perception in avian mate choice.

Keywords European serin · *Serinus serinus* · Carotenoid-based colouration · Female choice · Sexual selection · UV colouration

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A. V. Leitão · A. H. Monteiro · P. G. Mota
Department of Life Sciences, University of Coimbra,
Apt. 3046, 3301-401 Coimbra, Portugal

A. V. Leitão · A. H. Monteiro · P. G. Mota (✉)
CIBIO, Research Center in Biodiversity and Genetic Resources,
University of Porto, Campus Agrário de Vairão, 4485-661
Vairão, Portugal
e-mail: pgmota@uc.pt

Introduction

Conspicuous ornaments were used by Darwin (1871) as examples of traits that assumingly evolved under sexual selection. It is now widely accepted that the expression of elaborated ornaments such as colourful displays are sexually selected in many species (Andersson 1994; Hill 2006), exhibiting greater variability than naturally selected ones (Delhey and Peters 2008). In vertebrates, colouration results from structural colouration, interference in reflection and the deposition of melanin, carotenoids and other pigments (Hill and McGraw 2006). Sexual preference for carotenoid-based signals is now well established in birds (reviewed in Hill 2006), although there are exceptions (e.g. Pryke et al. 2001). The expression of carotenoid colouration can be considered an honest signal of individual quality used by females for mate choice (Hill 1999), since it is generally costly to produce and condition dependent (reviewed in Olson and Owens 1998). This type of colouration can be influenced by different factors, such as dietary access to pigments (McGraw 2006), moult speed (Serra et al. 2007) or parasite infections (Lozano 1994). Thus, it can be a reliable indicator of breeding performance (Serra et al. 2007), foraging ability (García-Navas et al. 2012), capacity to absorb and metabolise carotenoids (Hill 2000; McGraw et al. 2005), immunocompetence (Brawner et al. 2000; Saks et al. 2003b) or nutritional condition at the time of moult (Hill and Montgomerie 1994).

The perception of colours and the mechanisms of colour production have received great attention in studies with invertebrates and vertebrates (Tovée 1995; Hill and McGraw 2006) and, in particular, the significance of ultraviolet (UV) colouration, which is perceived by most birds (Cuthill et al. 2000b; Stevens and Cuthill 2007). UV reflectance may result from structural colours produced by the scattering of light and interference in the feathers, from pigment deposition (Shawkey and Hill 2005), and external influence like soiling

and preening (Zampiga et al. 2004) and by the application of uropygial oil (Pérez-Rodríguez et al. 2011, but see Delhey et al. 2008 for different results).

UV cues are known to be used in several circumstances such as in foraging behaviour (Church et al. 1998; Werner et al. 2012), navigation (Barta and Horvath 2004), regulation of circadian rhythms (Pohl 1992) and intra- and interspecific communication (Tovée 1995). Also, the interest in UV vision increased considerably due to its role in sexual signalling (Cuthill et al. 2000a; Johnsen et al. 1998; Siitari et al. 2002; Sirkiä and Laaksonen 2009; Griggio et al. 2010a), with two hypotheses being raised considering UV (1) as a special communication channel (Hausmann et al. 2003) or (2) as just part of the overall colouration without a specific signalling role (Hunt et al. 2001; Stevens and Cuthill 2007). Several studies demonstrated a condition dependence in UV colouration (in blue grosbeaks, *Guiraca caerulea*, Keyser and Hill 1999; blue tits, *Cyanistes caeruleus*, Sheldon et al. 1999; pied flycatchers, *Ficedula hypoleuca*, Siitari and Huhta 2002; black-capped chickadees, *Poecile atricapillus*, Doucet et al. 2005; and in budgerigars, *Melopsittacus undulatus*, Griggio et al. 2010b), suggesting that an individual variation in UV may be used in sexual signalling.

Most studies that tested the importance of UV in mate choice in birds were performed over structural colouration (Andersson and Amundsen 1997; Bennett et al. 1997; Liu et al. 2007), with less focus directed to UV reflectance associated with pigmented structures. However, most plumages based on carotenoid pigments also reflect in the UV due to the structural component of the feathers (Saks et al. 2003a), with pigments absorbing certain wavelengths that, otherwise, would be reflected by the feathers structure (Shawkey and Hill 2005; Shawkey et al. 2006; Jacot et al. 2010). There are other carotenoid-dependent sexual traits in birds that reflect in the UV, such as the combs of male red grouse, *Lagopus lagopus scoticus* (Mougeot et al. 2007), the bills of the zebra finch, *Taeniopygia guttata* (Hunt et al. 1997) or of mallards, *Anas platyrhynchos* (Peters et al. 2004), probably due to the reflecting properties of those tissues (Mougeot et al. 2007).

The importance of UV reflectance in carotenoid-based coloration was assessed in several studies (e.g. Mougeot et al. 2007; Jacot et al. 2010; Pérez-Rodríguez et al. 2011), but only a few were performed in the context of mate choice. In Pekin robins (*Leiothrix lutea*), males preferred partners viewed through UV-transmitting filter over those viewed through a UV-blocking filter (Maier 1993). Other studies measured and used multiple colour types without differentiating them; Hunt et al. (1999) showed that blue tits prefer to associate with UV-reflecting conspecifics, suggesting mutual mate choice for UV⁺; also, Bennett et al. (1996) showed that female zebra finches preferred males reflecting a full spectrum (UV⁺) over males with reduced UV.

The European serin is a sexually dichromatic cardueline finch, where males display a very conspicuous yellow carotenoid plumage (Stradi et al. 1995) which increases with age (Pagani-Núñez and Senar 2012), but for which, the female preference was not assessed before. In this study, we first tested for the existence of female choice over male yellow colouration in serins using the natural variation range. We then tested whether the UV component of colouration influences female choice in the same context, by using two different UV-blocking techniques — UV light filters and UV chemicals (Cuthill et al. 2000a) — to assess their potential role in female mate preference.

Methods

Two experiments were performed (experiments I and II) with two tests each (A and B) on a sample of wild captured birds. A total of 24 females and 33 males were used in experiment I (Exp I) in 2008 and other 12 females and 15 males in experiment II (Exp II) in 2011. There were no subjects in common between the two experiments.

Birds were captured outside the breeding season when still in flocks, near Coimbra, during the period January–March of 2008 and 2011 using unidirectional funnel cages and mist nets. Individuals were taken to and held in an indoor aviary in the Laboratory of Ethology of the University of Coimbra, Portugal. The aviary was under natural light with controlled ambient temperatures of 20±2 °C. Within the aviary, individuals were maintained in the same sex groups of six individuals in metal cages (118 cm×50 cm×50 cm), with perches and bathing water. Birds had ad libitum access to a commercial mixture of seeds (European Finches Prestige, Versele-Laga), mixed bird grit with crushed oyster and tap water, except during experiments. Sexes were visually but not acoustically isolated. After capture, each bird was banded with numbered black plastic rings (A. C. Hughes) for individual identification and was sexed and weighted (±0.5 g), and tarsus length (±0.01 mm) was measured. The male body condition was estimated from the unstandardised residuals of a linear regression of weight on tarsus. The relationship between the two variables was linear, with residuals over tarsus having an even distribution (Schulte-Hostedde et al. 2005). We also quantified ectoparasite load as described in Behnke et al. (1995). These parameters were analysed in relation to male colouration.

Spectral measurements

The colour reflectance of male serins was measured with a Ocean Optics USB4000 Spectrophotometer (Ocean Optics, Dunedin, FL, USA), with a deuterium and halogen light source (Mikropack Mini-DT-2-GS, UV–VIS–NIR), emitting between 300 and 700 nm, and an optical fibre reflectance

probe (Ocean Optics R400-7 UV–VIS) was used, which was held vertically and attached to a rigid black holder to standardise the distance between the probe and the sample (3 mm), providing a sampling area of 28 mm². All spectrum measurements were expressed in the proportion of light relative to a white standard (Ocean Optics, WS-1-SS White Standard). We took three readings for each sampled area. Each bird's plumage reflectance data was summarised by calculating tristimulus scores — brightness, hue and saturation — in visible (500–700) and UV wavelengths (320–420). The mean brightness (MBr) was calculated as the mean re-

fectance of the entire spectra $\left(MBr = \sum_{\lambda_{320}}^{\lambda_{700}} R_i/n_w \right)$, where

R_i is the reflectance at each wavelength class, and n_w is the number of wavelength classes. Ultraviolet mean brightness was calculated as the mean value of UV reflectance

$\left(\sum_{\lambda_{320}}^{\lambda_{420}} R_i/n_w \right)$, and hue as the wavelength reflection halfway

between the minimum and maximum reflection values (hue = $\lambda [R_{\max} + R_{\min}]/2$). Total saturation of the entire spectra was calculated as $(R_{\max} - R_{\min})/MBr$, and UV chroma, as the proportion of difference between UV maximum reflectance and minimum reflectance relative to mean brightness $(R_{\max_{UV}} - R_{\min})/MBr$ (formulas adapted from Montgomerie (2006) and Cardoso and Mota (2008)). Hue was excluded from the analysis, as it had coefficients of variation close to zero. We did not consider visual models for this study, although they can provide accurate measurements of colour perception by birds. However, they require parameters which are not always available and are taken through extrapolation from other species. As our experiments were performed in captivity with standardised illumination and background conditions and not under extreme light conditions, we considered appropriately to use tristimulus colour measurements.

The mean colour variables for each of the three yellow body regions (the throat, breast and belly) were calculated, and each colour variable was then averaged for the three regions. Finally, an ornamental colour score was calculated by performing a principal component analysis (PCA) using brightness, brightness UV, saturation and UV chroma variables. In Exp I, the first principal component (PC1) explained 52.80 % of the variation in colour among birds, and in Exp II, the PC1 explained 62.57 % of variation (Table 1). In both experiments, the males' PC1s had high factor loadings discriminating brightness (positive factor loads) and saturation (negative factor loads). Thus, we used PC1 to discriminate individuals with more saturated yellow colouration (M^+) from individuals with less saturated colouration (M^-). Saturation is positively correlated with carotenoid content of the feathers, as it was shown in other studies (Saks et al. 2003a; Butler et al. 2011). UV chroma did not differ between the two groups of males (Exp I, $F_{1, 32}=1.812$, $P=0.188$; Exp II, $F_{1, 14}=2.48$,

Table 1 Results of principal component analysis on reflectance variables for experiments I and II with factor loadings

	Experiment I		Experiment II	
	PC1	PC2	PC1	PC2
Mean brightness	0.810	-0.403	0.902	0.218
UV mean brightness	0.975	0.140	0.978	-0.204
Saturation	-0.698	-0.445	-0.519	0.837
Chroma UV	-0.137	0.881	0.681	0.642
% of variance	52.803	28.881	62.572	30.065

$P=0.139$). We used 17 M^+ and 16 M^- in Exp I and 8 M^+ and 7 M^- in Exp II. Males were paired for each mate choice trial, taking one from each group of more and less colourful.

Mate choice tests

The mate choice tests were performed between March and May in each year, during the morning, with at least 1 week of interval between the two sets of tests. The birds in captivity exhibited typical breeding behaviours, such as females gathering nesting material and males actively singing.

Tests were run in a three-compartment room (Fig. 1), which has the best performance in this kind of test, with low estimation errors (Bruzzone and Corley 2011). The walls were painted in white and had almost no reflectance in the UV

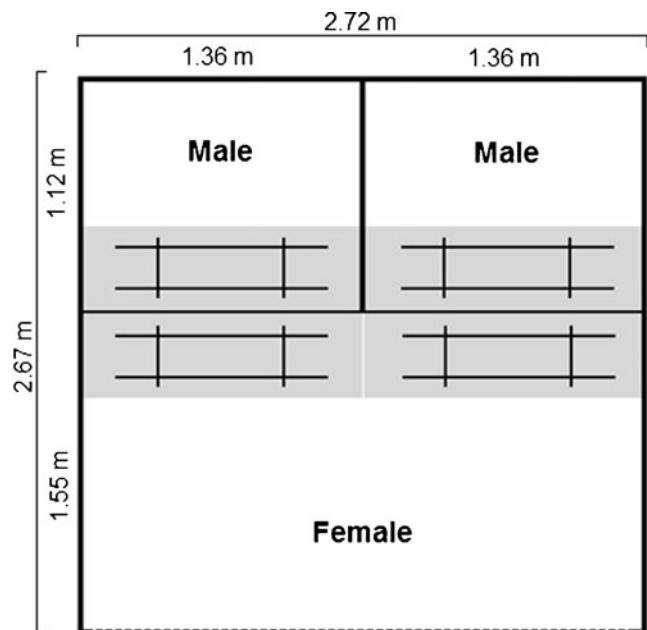


Fig. 1 Mate choice aviary with measurements. The height of the aviary was 2.00 m. The test apparatus was composed by three compartments: two for males and one for female, with a wall separating the males and a glass separating the female from both males. Video recording was made from behind the female compartment, through a wire mesh (dashed line). Choice areas are indicated by shading (136×40×8 cm), with perches represented

(only 5.6 % of total reflectance was in the UV region), while presenting a flat line reflectance peak ranging from 410 to 700 nm. The test room was illuminated by fluorescent lamps (Philips TLD 36 W/950) that have a spectral emission close to natural sunlight.

During each trial, the males were kept in two symmetrical and separate compartments with no visual contact between them. Male-type (M^+/M^-) position in relation to the female was consecutively changed to control for possible side effects. The female was introduced after the males into an adjacent compartment facing both males and separated from them by a glass. The closest area to each male's compartment in the female's compartment (represented in grey in Fig. 1) was designated as 'choice area', and the time spent there was used as a measure of female preference (Amundsen et al. 1997; Nolan and Hill 2004).

The trials lasted for 1 h, with the first 30 min for familiarisation to the new place, and the following 30 min was used for recording. All trials were recorded using the video camera Sony SSC-DC378P. Sound was also recorded to control for singing activity. The behaviours were analysed with the software Observer 5.1 and Observer XT (Noldus Information Technology). For both sexes, we measured the time spent in the choice area and number of shocks in the glass and, only in males, number of hops in the perches and number of songs. Singing activity and shocks were practically absent during the tests and were excluded from the analysis.

Experiment I

We performed two tests. Test A was performed to assess the female preference for male colouration (M^+/M^-) without manipulation of the UV (reflectance curves for more (M^+), and less colourful (M^-) males are represented in Fig. 2(a)). In test B, we assessed the importance of the UV component of male colouration on female choice, by manipulating the appearance of male birds by placing a UV light filter (as in Maier 1993; Bennett et al. 1996) (Perspex® VA Grade Sheet), in the glass separating the M^+ males ($M^+ UV^-$) from the female, with 98 % light transmittance. No filter was used in the M^- compartment, allowing a fully transmittance between 300 and 700 nm ($M^- UV^+$). Both compartments had similar light transmittance. We performed 24 mate choice trials in each test, using the same pairs of males in both tests. Each combination of two males was presented only once to females in each test.

Experiment II

We also performed two tests. Test A was identical to Exp I, and it was designed to assess the preference of females without manipulation of the UV of males. In test B, the importance of UV was assessed using a UV-blocking chemical

technique, similar to the one used in other studies (Sheldon et al. 1999; Siitari et al. 2002). In this test, the UV from M^+ males was directly reduced in each yellow body region (the throat, breast and belly) of their plumage ($M^+ UV^-$), by applying a UV-blocking chemical, while a neutral mixture was applied on the M^- ($M^- UV^+$). The treatments were applied with a cotton swab in all the coloured areas. The UV chemical applied was composed of a 75/25 % mixture of duck preen gland oil (CDC, Avonmore Tackle Products, Rathdrum, Wicklow, Ireland) and an UV-absorbing chemical (Eusolex 9020, Merck SA, Lisbon, Portugal). The neutral treatment was composed only of duck preen oil. The applied protocol took into account the results of persistence and temporal variation of the treatment (Korsten et al. 2007) which was previously assessed in a reduced sample of birds. There were no side effects of the treatments. The UV and neutral treatment effects were confirmed by spectrophotometry, as illustrated in Fig. 2(b, c).

We performed a total of 12 mate choice trials in each test, using the same pairs of males in both tests. Each combination of two males was presented only once to females in each test.

Statistical analysis

Generalised linear mixed models were initially performed to test for female association time with males, controlling for having used some males more than once in each test, although in a unique combination. Male identity was incorporated in the model as a random factor. As this factor did not have any explanatory value, and its removal had no effects on the results, we removed it and switched to generalised linear models (GLMs) with female time spent in the choice area as a dependent variable, the female as the subject variable and 'male type' (M^+/M^-) as within subjects' factor, for pair-wise comparisons. In order to assess any possible effects of males' behaviour on female choice, 'male activity' was also incorporated in the model as a predictor, constructed as the first component of a PCA of time in choice area and number of hops in perches (which explained more than 60 % of variance in all experiments and tests). The Wald chi-square statistic was used to test for significance in the GLMs. To check for female behavioural differences between the two consecutive tests, we performed paired *t* test for female time spent in the choice area between the tests A and B in each experiment. We tested for differences in males associated with colouration through one-way ANOVA, with colouration as a factor and male body condition and ectoparasites load as dependent variables.

All variables were normally distributed (Kolmogorov–Smirnov test), and variances were homogeneous (Levene statistic).

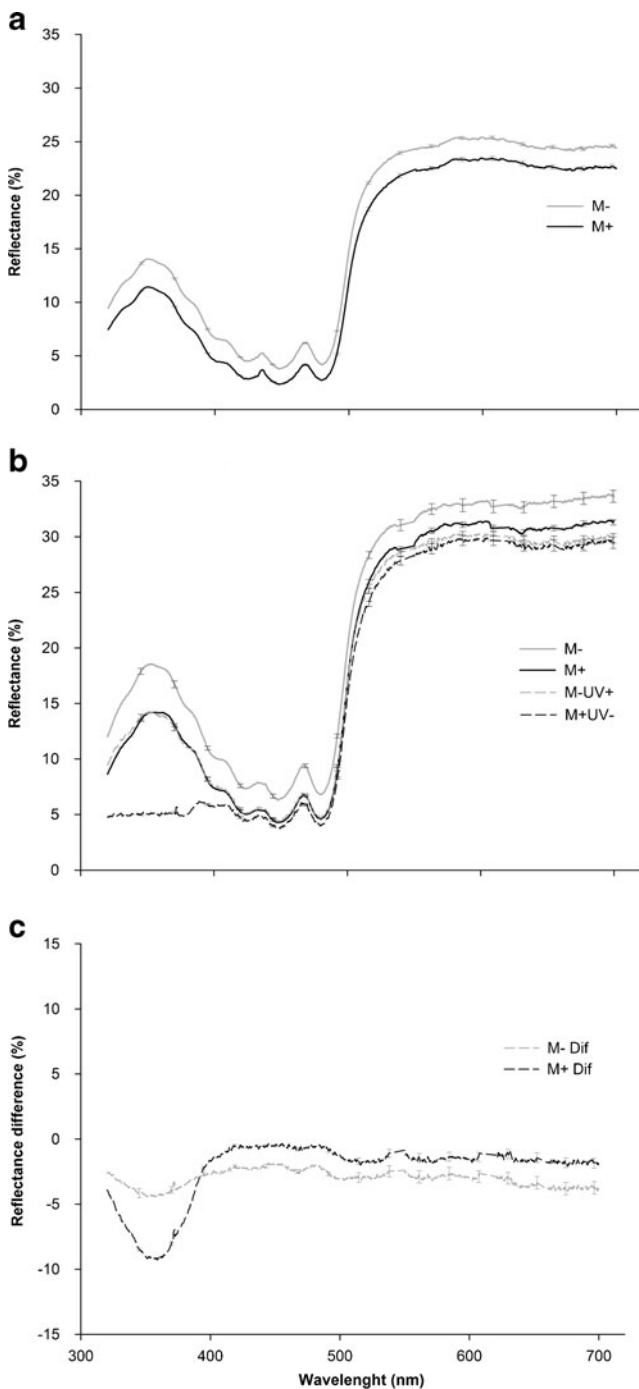


Fig. 2 Representation of reflectance spectra of males in experiments I and II. **a** The average reflectance spectra of more colourful (M^+) (black solid line) males and less colourful (M^-) (grey line) males in experiment I. **b** The average reflectance spectra of males before and after manipulation in experiment II. The black solid line represents M^+ males before treatment, the grey solid line, M^- males without treatment; the dashed grey line, the M^- males after the control treatment; and the black dashed line, the M^+ males with the UV treatment. **c** The difference in reflectance between treatments for M^+ (black dashed line) and M^- (grey dashed line) males in experiment II. Vertical bars denote SE at 21 nm intervals

Statistical analysis was performed with software IBM SPSS Statistics[®] 19.0 for Windows. All statistics are two-tailed.

Results

Females were highly motivated in both tests of each experiment, spending on average 93 % of their time in the choice areas. Also, female's activity was not affected by male's colour manipulation, as the total time spent in the choice areas was not different between the un-manipulated test and the manipulated ones in each experiment (experiment I, $t_{23}=0.626$, $P=0.537$; experiment II, $t_{11}=0.911$, $P=0.382$).

Experiment I

Females spent significantly more time in the choice area of M^+ males ($\chi^2_1=4.912$, $P=0.027$; see Fig. 3a) than of M^- males in the un-manipulated test (test A). When the UV filter treatment (test B) was applied, females stopped exhibiting any preference, as time spent in the choice areas was not significantly

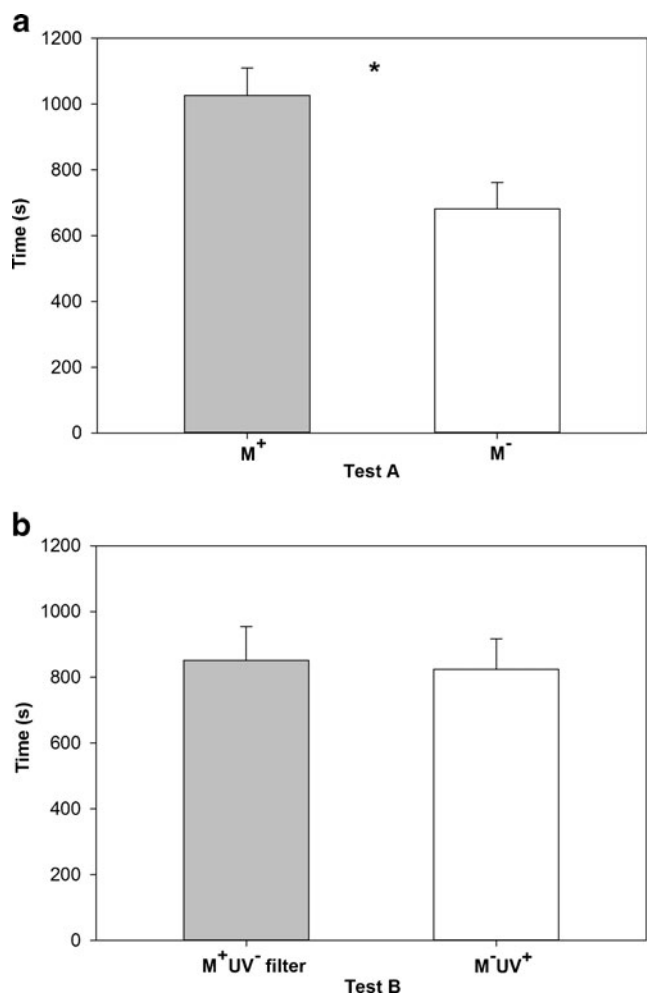


Fig. 3 Results of experiment I of time (in seconds) spent by females in front of each male. **a** Test A with the more colourful (M^+) and less colourful (M^-) males without manipulation. **b** Test B with the more colourful males with a UV filter (M^+UV^- filter) and less colourful males (M^-UV^+). Means \pm SE was represented ($N=24$); $*P<0.05$

different between male types ($\chi^2_1=0.021$, $P=0.885$, see Fig. 3b). Male activity did not influence female choice both in test A ($\chi^2_1=0.162$, $P=0.687$) and test B ($\chi^2_1=0.265$, $P=0.607$).

Experiment II

In test A, females spent significantly more time in the choice area of M^+ than in the area of M^- ($\chi^2_1=38.776$, $P<0.001$; see Fig. 4a). In test B, when we applied UV-blocking chemical to M^+ males and a neutral mixture to M^- males, females did not exhibit any preference, spending similar times in front of each type of male ($\chi^2_1=0.616$, $P=0.433$; see Fig. 4b). Male activity did not influence significantly female response in both tests (test A for male activity, $\chi^2_1=1.199$, $P=0.274$; test B for male activity, $\chi^2_1=1.903$, $P=0.168$).

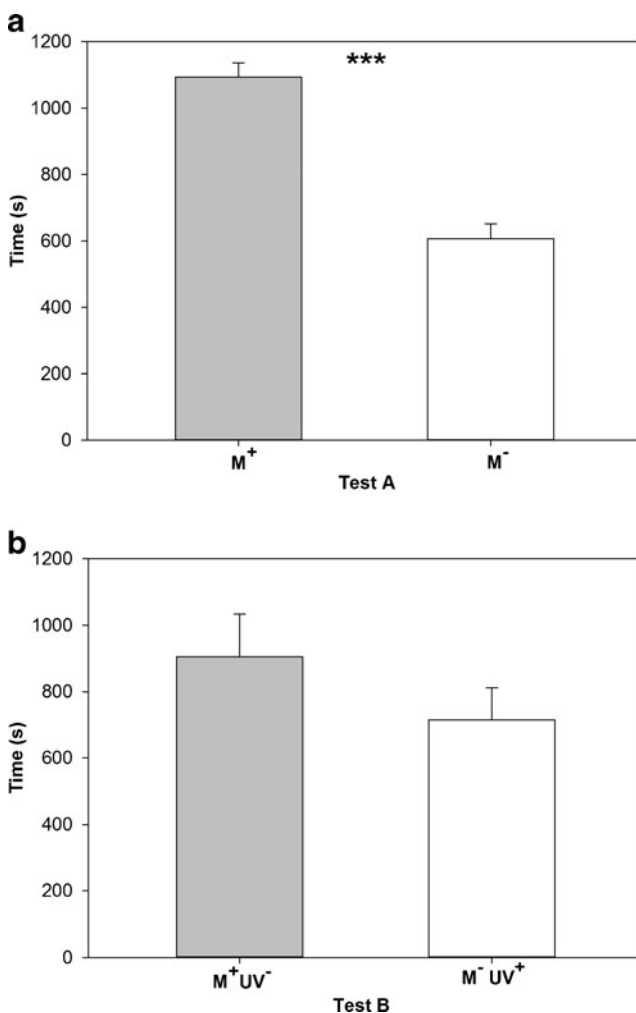


Fig. 4 Results of experiment II of time (in seconds) spent by females, in front of each male. **a** Test A with more colourful males (M^+) and less colourful males (M^-) without manipulation. **b** Test B with more colourful males with UV treatment (M^+UV^-) and less colourful males with control treatment (M^-UV^+). Mean \pm SE was represented ($N=12$); *** $P<0.0001$

Males' physical traits and colouration

There were no differences between males of the two categories (M^+/M^-) in ectoparasites load (Exp I, $F_{1,32}=1.982$, $P=0.169$; Exp II, $F_{1,14}=0.0$, $P=0.987$) and in body condition (Exp I, $F_{1,32}=3.402$, $P=0.075$; Exp II, $F_{1,14}=2.62$, $P=0.617$).

Discussion

We found the existence of female preference for male yellow carotenoid colouration in serins as females were more responsive to more colourful males in two different experiments. Also, females stopped exhibiting any preference for the more colourful males when the UV wavelengths were blocked.

Influence of carotenoid colouration in mate choice

The preference for carotenoid plumage colouration has now been demonstrated in several passerines, and it probably evolved as an honest signal of individual quality (reviewed in Hill 2006). Serins' plumage colouration, as in many other cardueline finches, is dependent on the deposition of carotenoid pigments in feathers (Stradi et al. 1995) which have to be incorporated in the diet. The results of our study showed that female serins preferred males with more yellow saturated colouration spending more time facing them. Time in close proximity is supported by several studies as a measure of preference (Amundsen et al. 1997; Nolan and Hill 2004) and seems to be correlated with the breeding success (Burley et al. 1994; Swaddle and Cuthill 1994). We concluded that females chose males with higher levels of carotenoids in their plumage as carotenoid concentration in plumage correlates directly with measurements of saturation (Saks et al. 2003a; Butler et al. 2011). Since the behaviour of males had no influence in female choice, we may infer that female serins were actually choosing the colouration of males. Also, there was no difference in morphological traits and ectoparasites load between more and less colourful males, which allows excluding these as possible confounding factors. Female serins may prefer more colourful males because this trait signals that they are in a better immunological condition or are better foragers, particularly important in a species where males also feed their broods. It was recently suggested that natural selection selects intermediate colouration phenotypes in male serins, through a long-term recapture data analysis (Figuerola and Senar 2007). The association found was with brightness but not with saturation, while no UV component was measured, which makes these results difficult to interpret in the present study. This selection for intermediate phenotypes could be the balanced result of different selective pressures with opposing effects acting on males, increasing breeding fitness against increasing

mortality, since more conspicuous individuals may be more susceptible to predation. Also, a higher investment in reproduction exhibited by some individuals might have negative effects on their survival.

Influence of UV reflectance in mate choice

The UV colouration is important in mate choice in birds, with several species showing preferences for ornaments reflecting UV over ornaments where UV reflectance was removed or reduced (Andersson and Amundsen 1997; Bennett et al. 1997; Hunt et al. 1997; Siitari et al. 2002; Sirkiä and Laaksonen 2009; Nolan et al. 2010, but see Ballentine and Hill 2003; Liu et al. 2007 for negative results). UV colouration can be indicative of phenotypic quality (Keyser and Hill 1999; Sheldon et al. 1999; Siitari and Huhta 2002; Doucet et al. 2005; Griggio et al. 2010b), supporting other evidence that UV may be a target of sexual selection.

Mate choice relative to UV reflectance in pigmented-based colouration has received much less attention than structural plumage colouration. Most of the studies manipulating UV reflectance to measure its importance in mate choice were performed in species where plumage colouration results from structural effects (Andersson and Amundsen 1997; Bennett et al. 1997; Liu et al. 2007). Regarding melanin-based colouration, in a study with pied flycatchers, males with increased UV were preferred by females, over males whose UV was reduced (Siitari et al. 2002). As to carotenoid-based pigmentation, except for the study by Maier (1993), no previous work was done to test the importance of UV in a mate choice context, through colour manipulation, albeit this type of colouration often has important reflectance in the UV (Saks et al. 2003a). Other manipulative studies attempted to measure the importance of UV in carotenoid traits, such as in blue tits (Hunt et al. 1999) or zebra finches (Bennett et al. 1996; Hunt et al. 1997), although in these works, multiple colour types and traits were studied simultaneously. In an interesting study of reproductive success measured in a natural population of yellowthroats (*Geothlypis trichas*), Freeman-Gallant et al. (2010) found that UV on carotenoid-based colouration was sexually selected conditional on male age. In another non-manipulative study, it was found that male rock sparrows (*Petronia petronia*) have a directional preference for female ornament size, but not for colouration including UV (Griggio et al. 2009).

Our results show that the blocking of UV reflectance has a negative effect on female's serin preference for colourful males and provide evidence that UV colouration is an important component of female choice in this species. Removal of the UV colouration from the most attractive males did not result in a choice shift towards less attractive males. This suggests that UV is not so relevant as to render attractive males completely unattractive or even be perceived as 'alien' males when UV is lacking. It is admissible that there were

other elements present or having greater expression in the most attractive males in the visible wavelengths, which levelled off the effect of blocking their UV reflectance. These results are compatible with both alternative hypotheses of UV colouration either as a special channel in communication among birds (Hausmann et al. 2003) or as just part of the light spectra of a sexual signal with no separate message content (Hunt et al. 2001; Stevens and Cuthill 2007). It is possible that UV reflectance is an inherent part of the signal, which results from the interaction between carotenoid absorbance and structural reflectance (Shawkey and Hill 2005; Shawkey et al. 2006; Jacot et al. 2010). In our sample of birds, there were no differences in morphology and ectoparasite load between more and less colourful males, but these results do not rule out the possibility that male colouration is associated with morphological traits in this species. These measurements were used to control for possible effects of morphological traits on mate choice of colouration, and they were not intended to assess the signalling value of colouration in male serins.

Most experimental testing of the importance of the UV reflectance in mate choice commonly makes use of UV light filters (Maier 1993; Bennett et al. 1996, 1997; Hunt et al. 1997) or UV-blocking chemicals directly on the plumage to reduce ultraviolet reflectance (Andersson and Amundsen 1997; Johnsen et al. 1998; Siitari et al. 2002), but the two techniques were never used in the same study before. A different experimental procedure was used in some studies by applying marker pens to the plumage (Ballentine and Hill 2003; Johnsen et al. 2005; Liu et al. 2007). This allows colour manipulation within the natural range of variation, although it has only been applied to structural plumage colouration, and it is not certain exactly how much the technique also affects other (longer) wavelengths where carotenoid pigments are most effective.

A series of studies have been conducted over blue tits' crown ornament, regarding the importance of UV reflectance, using several techniques to manipulate UV. By applying a UV-blocking chemical, it was demonstrated that male UV ornamentation affects offspring sex ratio (Sheldon et al. 1999), female's parental effort and offspring growth (Limbourg et al. 2004). Female parental adjustment was also found, when using marker pens (Johnsen et al. 2005). When seen through a UV-transmitting filter, male blue tits were preferred over individuals that had a UV-blocking light filter (Hunt et al. 1999). However, in a study by Kurvers et al. (2010) using marker pens to block the UV females did not discriminate UV-reduced males. In our study, both the UV-light filter and UV-blocking chemical were used in the same experimental design allowing for a direct comparison, and our results show that the outcomes are similar. Both methods have advantages and disadvantages. The use of filters prevents the physical manipulation of the individuals that can affect their

appearance in other ways. However, it changes the overall appearance of individuals, affecting other body parts that are not assessed in the study. In addition, it not only changes the bird's appearance, as it may also cause changes in the background, by greatly reducing the UV reflected and decreasing the contrast against the background. The manipulation of background contrast can affect the female's perception of the whole scene (Heindl and Winkler 2003), although it was suggested that a preference for UV may be context specific, not affecting the general preference for environments (Maddocks et al. 2001). However, the white walls of our test room had almost no reflectance in the UV, which means that the UV filter we applied had a very limited effect on the background, not reducing the perceived contrast.

The application of a blocking chemical on the bird's plumage has the advantage of not affecting the background and the bird background contrast, being more selective in the manipulation. Also, it does not change the male's perception of the female, as she is not affected by the treatment, but it is more invasive and can modify the subject's behaviour and other aspects of their physical appearance (Cuthill et al. 2000a). Our study showed that there were no differences in male's behaviour after applying the chemical. The combined results of the two experiments reveal that both techniques produced similar outcomes.

In conclusion, our study could determine that yellow carotenoid colouration is a sexually selected trait in male serins and that UV wavelengths are relevant in female mate choice, as UV blocking of plumage reduced male attractiveness. Our study also showed that the two techniques that were applied to block the UV (UV filter and UV chemical) produced similar results.

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Ethical standards This work was performed in accordance with the Portuguese National Authority for Animal Health (DGAV). Permits for animal capture, transport, maintenance, handling and experiments were carried out under the Institute for Nature Conservation and Biodiversity licences (63/2008/CAPT and 28/2011/CAPT) to PGM, according to Portuguese legislation.

Conflict of interest The authors declare that they have no conflict of interest.

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