Behav Ecol Sociobiol (2007) 61:741–751
DOI 10.1007/s00265-006-0304-z

ORIGINAL ARTICLE

Carotenoid-based colouration and ultraviolet reflectance of the sexual ornaments of grouse

F. Mougeot · J. Martínez-Padilla · L. Pérez-Rodríguez · G. R. Bortolotti

Received: 7 April 2006 / Revised: 17 October 2006 / Accepted: 28 October 2006 / Published online: 5 December 2006

Abstract Among the most familiar sexual signals are red, yellow, and orange sexual traits pigmented by carotenoids. Many birds can detect near-ultraviolet (UV) light, and UV signals can play key roles in mate choice. Grouse (Tetraonidae) exhibit bright carotenoid-dependent sexual ornaments, their supra-orbital combs, which to humans appear orange-red. Combs also reflect in the UV, which is not visible to humans but is likely to be visible to grouse. In male red grouse *Lagopus lagopus scoticus*, we show that comb UV reflectance decreases with increasing comb size and redness. By removing the epidermis of combs, where carotenoid pigments are, we show that the UV reflectance is a property of the dermis, underneath the red pigmented epidermis. Carotenoid pigmentation of combs acted as a mask to reduce reflectance by the dermis in the range 400–550 nm and in the UV, 300–400 nm. Patagium skin (non-ornamental skin under the wing) also reflects in the UV, but epidermis removal on this bare part tended to reduce UV reflectance, whereas removal of the red epidermis of combs increased UV reflectance. Males in better condition (greater body mass relative to size) had bigger and redder combs, but with less UV. Thus, carotenoid pigments of grouse combs are deposited on a white background with significant UV reflectance, which can influence how the signal is perceived by conspecifics. Carotenoid-based traits exhibit UV reflectance in a number of species, but how UV reflectance and carotenoid pigmentation influence colour remains little known for integumentary ornaments compared to plumage traits. UV vision is not uncommon in birds and other animals, so future studies should investigate how UV reflectance influences the perception of carotenoid-based signals of quality.

Keywords Carotenoid · Colour · Red grouse *Lagopus lagopus scoticus* · Sexual selection · Ultraviolet vision

Communicated by K. McGraw

F. Mougeot · L. Pérez-Rodríguez
Instituto de Investigación en Recursos Cinegéticos
(IREC, CSIC-UCLM-JCCM),
Ciudad Real, Spain

F. Mougeot · J. Martínez-Padilla
Centre for Ecology and Hydrology, Banchory,
Hill of Brathens,
Banchory, Aberdeenshire AB31 4BW, UK

F. Mougeot (✉)
School of Biological Sciences, University of Aberdeen,
Aberdeen, UK
e-mail: frm@ceh.ac.uk

G. R. Bortolotti
University of Saskatchewan,
Saskatoon, Canada

The brightly coloured ornaments exhibited by many bird species have been shown to function as reliable signals of quality, indicating better body condition or ability to resist parasites (e.g. Hamilton and Zuk 1982; Andersson 1994). Plumage colouration produced by feather structure and pigmentation by melanins or carotenoids has received particular attention (e.g. Hill and Brawner 1998; Badyaev and Hill 2000; Møller et al. 2000; Shawkey and Hill 2005), but birds also often possess brightly coloured fleshy ornaments (e.g. Bortolotti et al. 1996; Buchholz 1997). These ornaments, unlike feathers, can change colour within days (e.g. Burley et al. 1992; Bortolotti et al. 2003) and might thus be particularly important as indicators of current condition or health. Carotenoids determine the bright reds, yellows, and oranges of many sexual traits and are among the most familiar targets of female choice (Hill 2002).

Animals cannot synthesize carotenoids but must ingest them, so diet may ultimately limit ornament expression (Olson and Owens 1998; Hill et al. 2002). Carotenoids are also important antioxidants and powerful immuno-stimulants (Møller et al. 2000; Blount et al. 2003; Faivre et al. 2003). Individuals can allocate available carotenoids to ornaments or self-maintenance, and the resulting trade-offs may confer honesty on sexual signals (von Schantz et al. 1999; McGraw and Ardia 2003).

Many birds possess ultraviolet (UV) vision, and UV signals may play key roles in sexual signalling and mate choice (e.g. Bennett et al. 1996; Johnsen et al. 1998; Hunt et al. 1999; Cuthill et al. 2000). Recent studies have suggested a link between carotenoid-based colouration and plumage (structural) reflectance, and the spectra of carotenoid-pigmented ornaments often exhibit a secondary reflectance peak in the near UV (Burkhardt 1989; Bleiweiss 2004, 2005). There are also examples of traits other than plumage that are carotenoid-dependent and have significant UV reflectance, like the beaks of zebra finches *Taeniopygia guttata* (Bennett et al. 1996), blackbirds *Turdus merula* (Bright and Waas 2002) and mallards *Anas platyrhynchos* (Peters et al. 2004), the gape of passerine nestlings (Hunt et al. 2003), the combs of grouse (Mougeot et al. 2005a), and the cere of raptors (Mougeot and Arroyo 2006). How UV reflectance relates to carotenoid-based colouration has been studied for plumage traits (e.g. Bleiweiss 2004, 2005; Shawkey and Hill 2005) but remain largely unknown for integumentary ornaments.

Tetraonid birds (grouse family) exhibit brightly coloured supra-orbital combs that are particularly conspicuous sexual signals (Johnsgard 1983; Fig. 1a). These fleshy orange-red ornaments are pigmented by carotenoids (Hollett et al. 1984; Egeland et al. 1993). Combs also reflect in the UV (Mougeot et al. 2005a), and behavioural experiments have shown that grouse can see UV light (e.g. Siitari and Viitala 2002). In this study, we investigate the relationship between UV reflectance and carotenoid-based colouration of grouse combs and how both aspects relate to individual quality. We first show that the combs of four tetraonids (red grouse *Lagopus lagopus scoticus*, rock ptarmigan *L. mutus*, black grouse *Tetrao tetrix*, and western capercaillie *T. urogallus*) exhibit a bimodal pattern of reflectance, with a peak in the UV and, another, in the red part of the spectrum. In red grouse, we further analyse the relationships between male comb size and reflectance and test whether the UV reflectance is by the epidermal surface of combs, where lipid soluble carotenoids are present in lipid droplets (Hollett et al. 1984), or by the dermis, underneath the red epidermis. Finally, we test for condition-dependence in comb characteristics and investigate how sexual ornamentation (comb size and reflectance) relates to numbers of an important nematode parasite of red grouse.

Materials and methods

Data collection We sampled 60 red grouse that were shot for sport on Edinglassie estate, a grouse moor in NE Scotland, 12–27 August 2005. Grouse were sexed by plumage and ornaments (Cramp and Simmons 1980), and we randomly selected individuals from several dozen males shot on a given day. For each, we measured the maximum length (L) and width (W) of flattened combs (see Fig. 1a) with a ruler (nearest 1 mm) and calculated comb area (comb width×height) as a measure of ornament size (see Mougeot and Redpath 2004; Mougeot et al. 2005b). We weighed each bird (with a Pesola, to the nearest 5 g; males weighed 670±78 g; mean±SD, n=60) and measured wing length with a ruler (to the nearest 1 mm) as a measure of size and to calculate the condition index of body mass corrected for

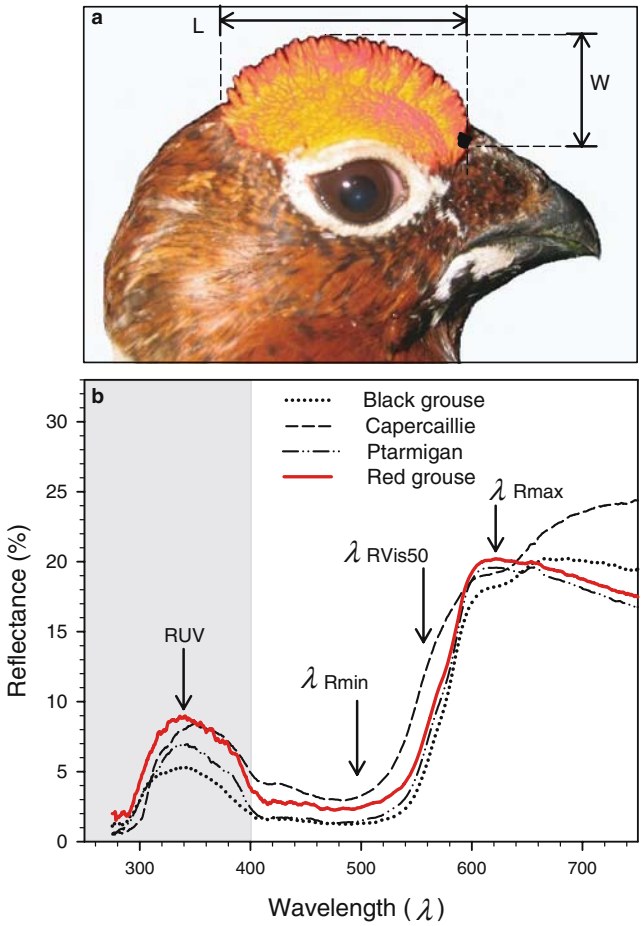

Fig. 1 **a** Portrait of a male red grouse showing the supra-orbital comb and the comb measurements (*L* maximum length and *W* maximum width); **b** reflectance patterns across the whole spectrum of light of the combs of males of four grouse species (black grouse *T. tetrix*, rock ptarmigan *L. mutus,* western capercaillie *T. urogallus*, and red grouse *L. lagopus scoticus*). The *grey area* represents the near-UV, invisible to humans

size (see Mougeot et al. 2005c). All measurements were taken by the same person (FM). After measurements, we cut and stored the combs at −20°C to later measure colour and removed a caecum and stored it in a cold room (at 4°C) to later count parasites.

Colour measurements Within 2 months of collection, we thawed the combs to measure their colour. Previous work showed that freezing the combs for this length of time did not affect the colour measurements (Mougeot et al. 2005a). We measured spectral reflection of the combs using a reflectance spectrometer. After thawing, the comb was illuminated using a deuterium-halogen light source (DH2000, Top Sensor System) with a spectral range from 280 to 800 nm. Comb reflectance was measured with a 45° to normal fibre-optic that provides illumination from the light source and transfers reflected light to the spectrometer (S2000). Measurements were done in a partially dark room, and the probe was placed against the comb surface, avoiding the effect of ambient light. We converted the data into digital information using a DAQ Card 700 and calculated reflectance data relative to a Spectralon® 99% white standard reference using the Spectrawin 3.1 software. Reflectance values were obtained at 0.4-nm intervals between 280 and 800 nm, enabling us to calculate the percentage reflectance at each interval point. We took two measures (one on each comb) for each male and used the average reflectance from both combs for analyses. Previous work showed that colour variables (see below) were repeatable within and between combs of the same bird (see Mougeot et al. 2005a).

Colour variables We summarized comb reflectance by calculating the following colour variables for each male in the interval 300–700 nm, which is the range of avian sensitivity (see Endler 1990): (1) total brightness (sum of reflectance in the interval 300–700 nm); (2) UV chroma (reflectance in the interval 300–400 nm, in percent, relative to total brightness); (3) red chroma (reflectance in the interval 600–700 nm, in percent, relative to total brightness); (4) UV spectral location or λ RUV (wavelength, λ, at which maximal reflectance was reached in the UV interval, 300–400 nm; see Fig. 1b); (5) comb redness, or λ Rvis50, as the wavelength of the reflectance midpoint in the visible interval (wavelength at which reflectance is halfway between its minimum, Rmin and its maximum, Rmax; see Fig. 1b). Spectral locations are measures that correspond to the human perception of hue. The total brightness, UV chroma and red chroma measures, relate to the amount of incident light that is reflected by the combs across the whole spectrum, in the UV and in the red, respectively.

Reflectance of bare parts with and without epidermis We investigated whether the UV reflectance was a product of the red epidermis of the combs or the dermis by carefully removing the red epidermis of combs with a scalpel. As a control, we also measured reflectance of the patagium with and without epidermis. This area of skin under the wing (triangular skin patch in between the arm bones) is the only area of bare skin (other than combs) found in red grouse and is not an ornament, as it has never been observed in visual displays. We removed the epidermis by slowly scraping the comb or surface of the patagium. For combs, the removal of the epidermis removed the red colouration. We used combs from 20 male red grouse and patagia from eight male red grouse. We measured reflectance with the spectrometer of intact combs or patagia (before manipulation) and of the same parts without the epidermis. For combs, we also measured the reflectance of the scraped red epidermis material placed on a black background.

Carotenoids in red grouse combs It is known from several grouse species that the visible colour of combs is due to carotenoid pigmentation (Hollett et al. 1984; Egeland et al. 1993), but this had not been established for red grouse. To ascertain that the red colouration of combs was also due to carotenoid pigments in our study species, we first used a simple chemical test that allows one to determine presence of carotenoid pigments (see McGraw et al. 2005a).

We also used high-performance liquid chromatography (HPLC) analysis to confirm that carotenoids were present in red grouse combs. The carotenoids were extracted with acetone following the procedures in Egeland et al. (1993). HPLC was carried out at the Molecular Ecology Laboratory of Doñana Biological Station using a Jasco PU-2089 Plus instrument equipped with a quaternary pump (Jasco Analítica Spain, S.L.). Carotenoid analyses were carried out using a reverse phase C18 column (Phenomenex Synergi, 4 μm) and a precolumn of the same material with a particle size of 5 μm. Samples were pre-filtered using an original equipment manufacturer filter Nylon (0.45–4 mm) and later injected with a Rheodyne 7725i Valve equipped with a 20-ml loop (Rheodyne, Rohnent Park, CA, USA). The eluent system was as the one described in Mínguez-Mosquera (1993) except that the flow rate was 1 ml min^{-1}. Data were acquired between 195 and 650 nm with a multiwavelength detector MD-2010 Plus (Jasco Analítica Spain, S.L.).

Quantification of carotenoids was performed using reference cantaxanthin and lutein. Known dilutions of both reference pigments were injected in the HPLC instrument to build a calibration curve at 450 nm. Concentration of individual carotenoids was calculated from HPLC areas recorded at 450 nm (see Negro et al. 2001). We analysed combs from five different males, each sampled twice.

Parasite counts The caecal threadworm *Trichostrongylus tenuis* has a direct life cycle with no alternate host. *T. tenuis* is known to have negative effects on the condition, energy, fecundity, and survival of red grouse (Hudson 1986; Delahay et al. 1995). Within 2–5 days after collection, we estimated the number of nematodes per host using caecal egg counts for 58 males (see Seivwright et al. 2004). *T. tenuis* caecal egg concentration provides a reliable estimate of the number of worms per host (Seivwright et al. 2004).

Statistical analyses We used SAS 8.01 (SAS, Statistical Analysis System) for all analyses. When explaining variation in condition, we fitted (log transformed) body mass to models and included wing length as a fixed effect (see Darlington and Smulders 2001; García-Berthou 2001). We calculated parasite load (number of worms per grouse) from caecal egg concentration using equations in Seivwright et al. (2004) and log-transformed parasite data for all analyses. We used the Princomp procedure (SAS 2001) for the principal components analysis of comb characteristics (size and all colour variables detailed above). We used *t*-tests for paired samples for differences in reflectance of combs or patagia before and after the removal of the epidermis. All tests are two tailed, and all data are given as mean±SD.

Results

Reflectance pattern of grouse combs Figure 1b shows the reflectance patterns of combs of four tetraonids. All showed a bimodal pattern, with a peak in the UV (300–400 nm) and in the red (600–700 nm). We further investigated comb reflectance variation in red grouse. In this species, males with bigger combs had redder combs (greater λ Rvis50 and red chroma), but UV chroma was negatively related to λ Rvis50 and to comb size (Table 1). We further conducted a principal components analysis of comb characteristics. The first axis explained 41% of variation, with comb size, λ Rvis50, red chroma, and total brightness having the highest positive loadings. The second axis explained a further 34% of variation, with UV chroma and total brightness having the highest positive loadings (Table 1). The principal components analysis confirmed a contrast between comb size and redness, and UV reflectance.

Carotenoids in red grouse combs Chemical tests (McGraw et al. 2005a) confirmed that carotenoids were present in the combs of red grouse (n=3 samples tested, all positive) and, in particular, in the red epidermal surface of combs (n=3 samples tested, all positive). The HPLC analyses also confirmed that combs were pigmented by carotenoids (Fig. 2). These included lutein, astaxanthin, and seven other red carotenoid pigments of cenotic type (Table 2). Canthaxanthin was not found in red grouse combs.

Carotenoid pigmentation and UV reflectance The principal components analysis' results and the negative relationship between UV chroma and comb redness suggested an antagonism between carotenoid pigmentation and UV reflectance. We evaluated this further by testing whether UV reflectance of combs was a property of the red pigmented epidermis or the dermis of combs. By removing the red epidermal surface of combs and comparing comb reflectance with and without this layer, it appeared that UV reflectance was by the dermis of combs, which was pale and had little colour (Fig. 3a). UV chroma by the dermis of combs (without red epidermal)

Table 1 Correlations between comb size and comb reflectance variables (Pearson correlations; n=60 males; significant correlation coefficients, at the P=0.05 level, are highlighted in bold) and results of principal components analysis on size and reflectance variables

	λ Ruv	λ Rvis50	UV chroma	Red chroma	Total brightness	Principal components		
						First component	Second component	Third component
Comb size	+0.001 (ns)	**+0.606** (<0.001)	**−0.399** (<0.01)	**+0.599** (<0.001)	+0.164 (ns)	0.456	−0.374	−0.058
λRuv		+0.027 (ns)	+0.183 (ns)	+0.215 (ns)	**+0.289** (<0.05)	0.195	0.246	0.946
λRvis50			**−0.379** (<0.01)	**+0.516** (<0.001)	0.089 (ns)	0.419	−0.385	0.036
UV chroma				+0.102 (ns)	**+0.719** (<0.001)	0.059	0.656	−0.216
Red chroma					**+0.757** (<0.001)	0.604	0.050	−0.114
Total brightness						0.457	0.468	−0.199
Eigenvalues						2.46	2.04	0.87
Variance explained								
—by each component						41.0%	34.1%	14.5%
—cumulative						41.0%	75.1%	89.6%

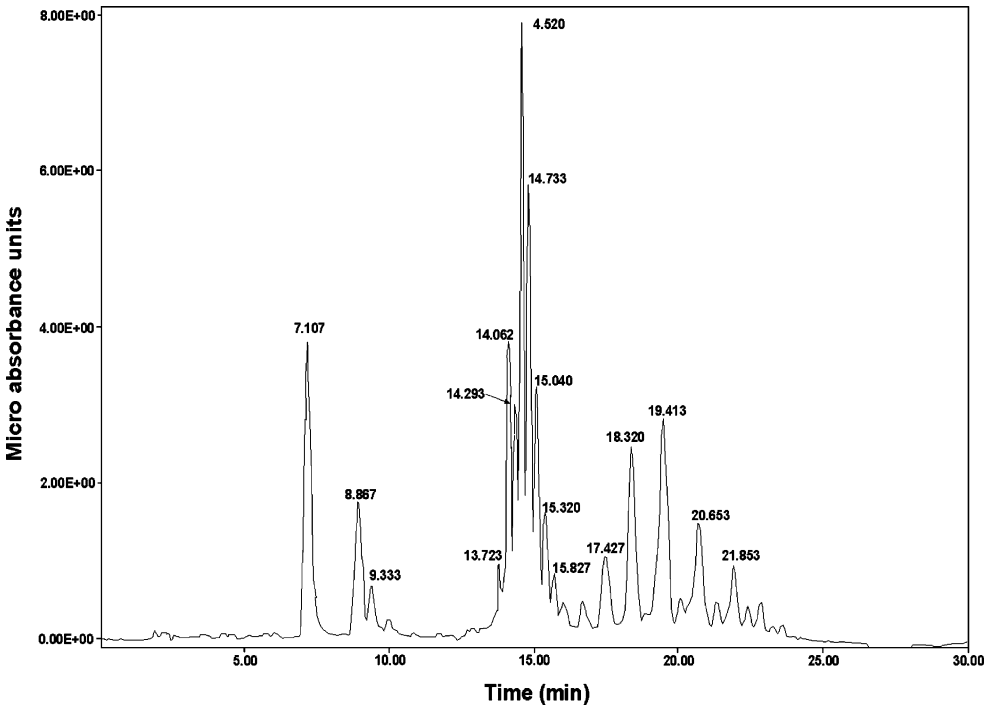

Fig. 2 Chromatogram of red grouse comb carotenoid pigments (*X*-axis time, in minutes; *Y*-axis micro-absorbance units) obtained from HPLC (see Materials and methods). Values at peaks refer to retention times

was always greater than that of intact combs (*t*-test for paired samples: $t_{1,19}$=6.30; P<0.001; mean±SD difference in UV chroma of 6.9±3.7%; n=20; Fig. 3a).

The patagium was pale with little colour and also exhibited significant UV reflectance (peak in reflectance in the interval 300–400 nm; Fig. 3b). When removing the epidermis of the patagium, the patagium dermis underneath also showed UV reflectance (Fig. 3b). Unlike combs, however, UV chroma of the patagium dermis (without epidermis) tended to be lower (not higher) than that of intact patagia (mean±SD difference in UV chroma of −3.2 ±5.5%; n=8; Fig. 3b), the difference being not statistically significant (*t*-test for paired samples: $t_{1,7}$=0.67; P>0.10).

We further tested whether the difference in UV chroma between intact comb and dermis (without the red epidermis) varied with comb redness of intact combs. If carotenoid pigments act as a mask to UV reflectance by the dermis, then, increased epidermal pigmentation of combs (redness) should be associated with a greater reduction in UV reflectance. Accordingly, we found that the difference in UV chroma between intact combs and dermis increased with increasing λ Rvis50 ($F_{1,18}$=17.39; P<0.001) and red chroma ($F_{1,18}$= 8.55; P<0.01) of intact combs. In contrast, the difference in UV chroma between intact combs and dermis was not significantly related to λ RUV ($F_{1,18}$=3.33; P=0.09), UV chroma ($F_{1,18}$=0.60; P=0.44), or total brightness ($F_{1,18}$= 0.00; P=0.94) of intact combs.

Condition and ornamentation We tested whether variation in male body condition could be explained by comb

Table 2 Results of the HPLC analyses (carotenoid concentration, in microgram per gram) conducted on five male red grouse combs

Sample	Lutein	Astaxanthin	Red 1	Red 2	Red 3	Red 4	Red 5	Red 6	Red 7	All
Comb 1a	6.68	17.47	3.44	66.86	3.49	6.51	8.80	3.12	0	116.36
Comb 1b	6.65	16.71	2.98	67.05	2.84	6.35	8.63	3.19	0	114.40
Comb 2a	18.52	45.17	6.35	116.57	6.53	10.11	10.62	4.37	0	218.25
Comb 2b	18.86	46.06	6.37	118.78	6.41	10.24	10.72	4.47	0	221.90
Comb 3a	16.89	34.88	6.43	146.14	13.32	23.97	32.52	18.91	9.14	302.22
Comb 3b	16.66	34.19	6.59	139.84	12.80	23.91	32.07	18.92	9.08	294.06
Comb 4a	16.15	31.93	5.28	129.96	9.90	20.46	33.85	18.62	7.69	273.83
Comb 4b	16.98	33.04	4.99	147.14	9.02	21.41	35.54	20.12	8.80	297.04
Comb 5a	8.84	21.19	3.29	65.63	2.96	6.67	9.02	4.82	1.79	124.22
Comb 5b	8.76	20.99	3.36	62.43	3.00	6.34	8.42	4.32	1.75	119.37

Each comb was sampled twice. Pigments Red 1–7 were red carotenoids of cenotic type that were not identified.

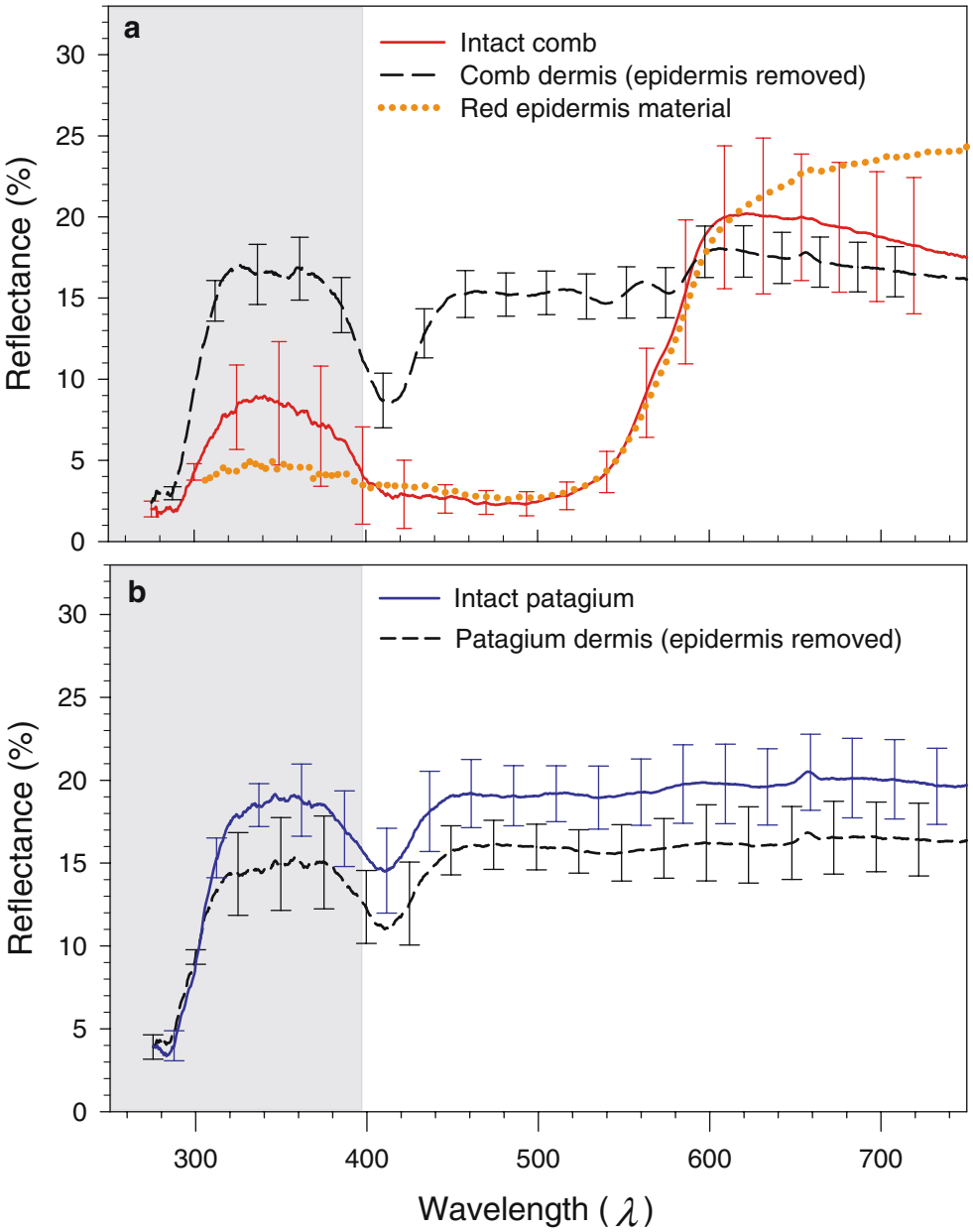

Fig. 3 Reflectance patterns of comb (**a**) and patagium (**b**) before (intact comb or patagium) and after removal of the epidermis (comb or patagium dermis). For grouse combs, (**a**) shows the average reflectance of the intact combs (*solid red line*, n=20) of the dermis of comb (after the removal of the red epidermis; *dashed line*, n=20) and of the removed red epidermis material placed on a black surface (*dashed orange line*, n=10). For patagium skin, (**b**) shows the average reflectance of the intact patagium (*solid blue line*, n=8) and of the patagium dermis (after the removal of the epidermis; *dashed line*, n=8). The *grey area* represents the near-UV, invisible to humans. *Error bars* represent standard errors

characteristics. Body mass variation was significantly explained by wing length ($F_{1,59}$=36.99; P<0.001), so we included wing length as a fixed effect in all models to correct body mass for size (condition index). Univariate analyses indicated that variation in male condition was explained by comb size and red chroma but was not significantly explained by λ RUV, λ Rvis50, UV chroma, or comb brightness (Table 3). Males in better condition had bigger combs with greater red chroma and tended to have less UV bright combs.

We also tested whether variation in male condition could be predicted from comb characteristics as summarized by principal components analysis (Table 1). After controlling for wing length, variation in body mass was significantly explained by PC1 (positive correlation; Fig. 4) and PC2 (negative correlation), but not PC3 (Table 3). This confirmed that better condition can be predicted from bigger, redder combs (PC1) with less UV reflectance (PC2).

Parasites, condition, and sexual ornamentation Males had, on average, 951 *T. tenuis* worms (range 0–6,034 worms; n=58). Parasite load was not related to body mass ($F_{1,57}$=0.77; P=0.39), but male condition (mass corrected for size) was significantly positively related to *T. tenuis* load (Table 3). Univariate analyses showed that *T. tenuis* load had a significant positive correlation with comb size and negatively correlated with UV chroma but was not significantly related to red chroma, λ RUV, λ Rvis50, or total brightness (Table 3). When testing whether parasite

Table 3 Summary statistics for the regression analyses (generalized linear models) of condition (mass corrected for size) and number of *T. tenuis* parasites on comb characteristics (univariate analyses on each comb variables, and regression on the first three axis, PC1–3, of the principal components analysis on comb characteristics; see Table 1)

Regression of *X* on *Y*	Parameter estimate (slope±SE)	*F* value	*df*	*P* value
Condition[a]				
Comb size	0.81±0.12	46.76	1.59	<0.001
Comb λ RUV	0.54±1.46	0.14	1.59	0.71
Comb λ Rvis50	1.21±0.76	2.51	1.59	0.12
Comb UV chroma	−5.60±2.97	3.56	1.59	0.06
Comb red chroma	6.68±2.06	10.51	1.59	<0.01
Comb brightness	3.53±4.08	0.75	1.59	0.39
PC1	19.61±4.62	18.03	1.55	<0.001
PC2	−13.04±4.58	8.10	1.55	<0.01
PC3	1.77±6.78	0.07	1.55	0.79
Parasite load[b]				
Comb size	0.016±0.005	7.13	1.57	<0.01
Comb λ UV	0.052±0.064	0.65	1.57	0.43
Comb λ Rvis50	0.034±0.031	1.27	1.57	0.26
Comb UV chroma	−0.350±0.124	7.92	1.57	<0.01
Comb red chroma	0.059±0.096	0.37	1.57	0.54
Comb brightness	−0.215±0.181	1.42	1.57	0.24
PC1	0.302±0.208	2.09	1.54	0.15
PC2	−0.371±0.180	4.25	1.54	<0.05
PC3	0.541±0.334	2.63	1.54	0.11
Condition[a]				
Parasite load[b]	5.95±2.93	4.13	1.57	<0.05

[a] The dependent variable is body mass (in grams), and wing length (millimetre) was included as a fixed effect in all analyses to analyse variation in body mass corrected for size (condition index).
[b] Parasite load (number of *T. tenuis* worm per grouse) was log-transformed ($\log_{10}+1$) for all analyses.
Significant ($P<0.05$) predictors are highlighted in bold.

load could be predicted from comb characteristics, we found that variation in *T. tenuis* load was not significantly explained by PC1 or PC3 but was explained by PC2 (negative correlation; Table 3). Thus, more worms were predicted for males with larger and redder combs but with less UV reflectance.

Discussion

Carotenoid pigmentation and UV reflectance

UV signals can play key roles in social and sexual signalling, with males showing greater UV reflectance being preferred (Johnsen et al. 1998; Hunt et al. 1999; Cuthill et al. 2000). In this study, we have shown that UV reflectance by a sexual ornament may not be independent of its carotenoid-based pigmentation. This is important, because carotenoid-based signals of quality are amongst the commonest in birds, and their background reflectance, and in particular UV reflectance, can influence how the signals are perceived.

Recent work has shown that the colour of carotenoid-bearing feathers is created both by reflection of light from white structural tissue and absorption of light by carotenoids (Shawkey and Hill 2005). The fleshy ornaments of grouse have an overall pale (white) background with significant UV reflectance, and the epidermal carotenoid pigmentation modifies this reflectance by absorption at short visible wavelengths and in the near UV. The latter, most likely, influences how receivers possessing UV vision would perceive comb colour. Experiments have demonstrated that black grouse can distinguish slight differences in reflectance in the UV range and use UV vision for foraging (Siitari and Viitala 2002). Given the sensitivity of the UV cone of birds of the order Galliformes, to which grouse belong, the red grouse should also be capable of perceiving the UV variation in comb reflectance (Siitari and Viitala 2002).

Male grouse with bigger and brighter combs are typically more aggressive and preferred by females (e.g. Bart and Earnst 1999; Rintamaki et al. 2000; Redpath et al. 2006). Combs are raised during social encounters as a result of increased blood flow in comb capillaries (Hollett et al. 1984). However, the intensity of visible colouration does not increase when the combs are raised (see Hollett et al. 1984), unlike the wattles of domestic fowl and red jungle fowl *Gallus gallus* (Zuk et al. 1995). Thus, different mechanisms are involved in grouse comb erection and colouration (Hollett et al. 1984). The orange-red colour of combs of western capercaillie, blue grouse *Dendragapus obscurus*, and spruce grouse *Falcipennis canadensis* is due to carotenoid pigmentation (Johnsgard 1983; Hollett et al. 1984), and our tests confirmed the same for red grouse. The red colour of grouse combs originates from a zone of lipid material within and directly below the epidermal surface, where lipid-soluble carotenoids are present in lipid droplets (Hollett et al. 1984). In western capercaillie, the main carotenoids of combs have been identified as astaxanthin

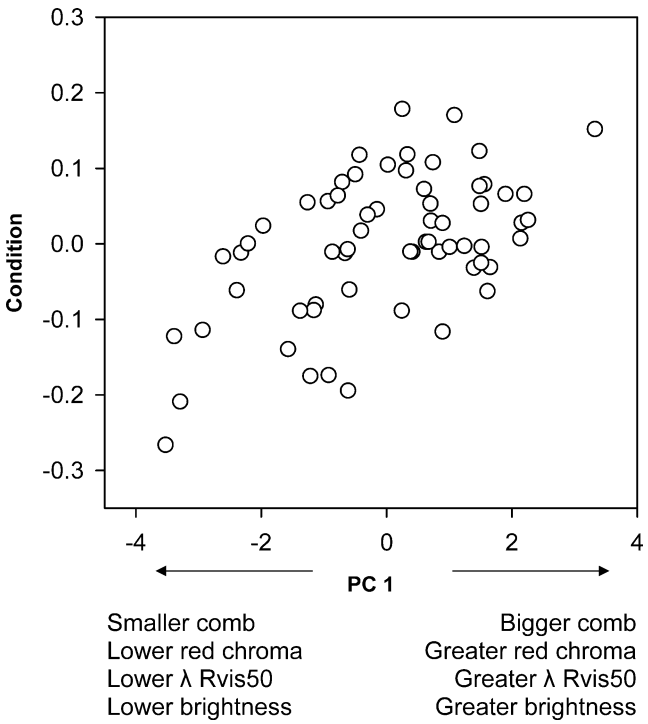

Fig. 4 Condition-dependence of comb characteristics as summarized by the first axis of a principal components analysis on comb size and reflectance (see Table 1). Male conditions are residuals from generalized linear models of log(body mass) on log(wing length)

and zeaxanthin, with also traces of adonixanthin and lutein (Egeland et al. 1993). In red grouse combs, we also found that astaxanthin and lutein pigments were present, as well as seven other red carotenoid pigments of cenotic type (Table 2). Canthaxanthin, which is commonly found in birds, was not present in red grouse combs. Our results confirmed that red grouse combs were also pigmented by carotenoids.

Mechanisms underlying UV reflectance by non-plumage (structural) ornaments remain little known compared to those of plumage. In tanagers (Thraupini, Passeriformes), the bimodal reflectance pattern of carotenoid bearing feathers is the result of a strong absorption at short visible wavelengths (380–550 nm) by carotenoids, with plumage structure influencing the degree to which UV is reflected (Bleiweiss 2004, 2005). In grouse, the carotenoid pigmented surface of combs strongly absorb reflectance in the same interval (400–550 nm), and also, but to a lesser extent, in the UV interval (300–400 nm; see Fig. 3a). The reduction in UV chroma between dermis and pigmented combs decreased with increasing comb redness, showing that the carotenoid pigmentation acts as a mask that reduces UV reflectance by the dermis of combs. We could not distinguish between absorbance by pure carotenoid pigments and that by the pigments within the epidermal cells, so future work could investigate how the carotenoids extracted from combs absorb light and align this with the reflectance spectra obtained from intact combs and their background.

UV reflectance was not restricted to combs, as we also found it in another area of bare skin, the patagium underneath the wing. More work is needed to understand how the dermis reflects in the UV, and future studies could look for UV reflecting structures in the dermis of grouse combs similar to those recently discovered in the beak of *Aptenodytes* penguins (see Dresp et al. 2005). As the patagium is not displayed and does not function as a signal, it is unlikely that the UV reflectance by the combs of grouse evolved as a signal in itself. Depositing red carotenoid pigments on an overall white and UV reflective surface might nevertheless have evolved as a way to enhance the conspicuousness of the signal. For humans, who do not see UV light, red against white provides a striking contrast. For birds with UV vision, a UV reflecting background might further influence perception: low quality individuals, with less carotenoids available for colouration, would leave more uncovered UV reflective surface that would better expose this lack of pigmentation. Another possibility is that carotenoids were allocated to bare parts as a way of protecting skin from photo damage, and that the carotenoid-based colouration of these bare parts subsequently evolved as a signal of quality (Bortolotti 2006). Carotenoids have photoprotective properties (Wynn-Williams and Edwards 2002) and have been shown to protect skin against UV light-induced erythema (Stahl and Sies 2002; Aust et al. 2005).

Comb size and reflectance as honest signals of quality

Honest signals of individual quality should be condition dependent, so that only individuals in prime condition exhibit the biggest or brightest ornaments (Andersson 1994). Accordingly, we found that males in better condition had bigger and redder combs. Carotenoid-dependent ornaments might be honest indicators of quality because animals cannot synthesize carotenoids and only good foragers ingest enough carotenoids to show bright colour (Hill et al. 2002). Thus, birds in better condition would have more carotenoids to deposit in their ornaments. Red grouse feed almost exclusively on heather *Calluna vulgaris*, a plant of poor digestibility, so grouse have particularly long caeca to maximize digestion (Moss 1972). *T. tenuis* worms, which inhabit the caeca, also damage to the caecal walls (Seivwright et al. 2004) and could interfere with carotenoid absorption and, thereby, directly reduce carotenoid availability. Moreover, carotenoid absorption and metabolism can be costly (Hill 2000; McGraw et al. 2005b), and individuals in poor body condition might have a lower capacity to acquire carotenoids and to transform them into the specific carotenoid pigments allocated to ornaments. Because carotenoids are antioxidants and can

directly boost the immune system, carotenoid-based colour signals in birds may also directly signal male health (von Schantz et al. 1999; McGraw and Ardia 2003). Diseased and parasitized individuals would allocate fewer carotenoids to their ornaments because they need them as antioxidants and for resisting parasites (e.g. Thompson et al. 1997; Brawner et al. 2000).

The allocation of carotenoid pigments to ornaments may be modulated by testosterone (Mundinger 1972; Owens and Short 1995; McGraw et al. 2006). In red grouse, testosterone enhances both comb size (Mougeot et al. 2004, 2005b) and redness, as bigger combs are also redder (this study). Previous studies have shown no significant correlation between comb size and *T. tenuis* load in male red grouse (Mougeot et al. 2004; Mougeot and Redpath 2004). However, experiments have shown that testosterone and parasites interact in two ways in red grouse: elevated testosterone increases *T. tenuis* load (Seivwright et al. 2005) by increasing host susceptibility (Mougeot et al. 2005d), and high parasite intensities can limit the expression of testosterone-dependent comb size (Mougeot et al. 2005c). Males in better condition had more *T. tenuis* parasites and had bigger and redder combs but with less UV reflectance, which was unexpected. Males with bigger and redder combs might have been investing more in sexual or territorial activities to the detriment of their parasite defences, which could explain the positive relationship we found. Males might be able to do so when parasite intensities are low and do not impact on condition, as found here (average<1,000 worms). At higher parasite abundances, we would expect males with more parasites and in poorer condition to show less carotenoid pigmentation and relatively more UV reflectance.

We showed that combs are complex sexual signals, with a carotenoid-based pigmentation deposited on an overall white background with UV reflectance. Whether UV signals are special has been both advocated and criticized (Banks 2001; Hausmann et al. 2003), but the role of the UV waveband should be considered together with the rest of the avian visible spectrum (Hunt et al. 2001). The UV reflectance of grouse combs may influence how this carotenoid-based signal is perceived.

Acknowledgement We are grateful to the owner and gamekeepers from the Edinglassie estate for allowing us to collect the data on red grouse. Particular thanks are due to D. Calder for his help with the data collection and to R. Moss for providing specimens of black grouse, capercaillie, and ptarmigan for comb reflectance measurements. We are very grateful to I. Garcia and J. J. Negro (Molecular Ecology Laboratory of Doñana Biological Station, CSIC Spain) for conducting the HPLC analyses on red grouse combs. We also thank M. D. Padilla for transporting samples and C. Alonso-Alvarez for helpful comments on the manuscript. F. Mougeot was supported by a Ramon y Cajal grant (Ministerio de Educación y Ciencia, Spain). L. Pérez-Rodríguez was supported by a pre-doctoral FPU grant (Ministerio de Educación y Ciencia). J. Martínez-Padilla was supported by a post-doctoral grant (ref. EX-27-04-04, Ministerio de Educación y Ciencia). G. Bortolotti was supported by a NSERC grant.

References

Andersson M (1994) Sexual selection. Princeton University Press, Princeton, NJ

Aust O, Stahl W, Sies H, Tronnier H, Heinrich U (2005) Supplementation with tomato-based products increases lycopene, phytofluene, and phytoene levels in human serum and protects against UV-light-induced erythema. Int J Vitam Nutr Res 75:54–60

Badyaev AV, Hill GE (2000) Evolution of sexual dichromatism: contribution of carotenoid- versus melanin-based coloration. Biol J Linn Soc 69:153–172

Banks AN (2001) For your eyes only? The role of UV in mate choice. Trends Ecol Evol 16:473–474

Bart J, Earnst SL (1999) Relative importance of male and territory quality in pairing success of male rock ptarmigan (*Lagopus mutus*). Behav Ecol Sociobiol 45:355–359

Bennett ATD, Cuthill IC, Partridge JC, Maier EJ (1996) Ultraviolet vision and mate choice in zebra finches. Nature 380:433–435

Bleiweiss R (2004) Novel chromatic and structural biomarkers of diet in carotenoid-bearing plumage. Proc R Soc Lond B 271:2327–2335

Bleiweiss R (2005) Variation in ultraviolet reflectance by carotenoid-bearing feathers of tanagers (Thraupini: Emberizinae: Passeriformes). Biol J Linn Soc 84:243–257

Blount JD, Metcalfe NB, Birkhead TR, Surai PF (2003) Carotenoid modulation of immune function and sexual attractiveness in zebra finches. Science 300:125–127

Bortolotti GR (2006) Natural selection and avian coloration: protection, concealment, advertisement or deception? In: Hill GE, McGraw KJ (eds) Avian coloration, vol 2: function and evolution. University Press, Harvard, pp 3–35

Bortolotti GR, Negro JJ, Tella JL, Marchant TA, Bird DM (1996) Sexual dichromatism in birds independent of diet, parasites and androgens. Proc R Soc Lond B 263:1171–1176

Bortolotti GR, Kimberley JF, Smiths JE (2003) Carotenoid concentration and coloration of American kestrels (*Falco sparverius*) disrupted by experimental exposure to PCBs. Funct Ecology 17:651–657

Brawner WR, Hill GE, Sundermann CA (2000) Effects of coccidial and mycoplasmal infections on carotenoid- based plumage pigmentation in male house finches. Auk 117:952–963

Bright A, Waas JR (2002) Effects of bill pigmentation and UV reflectance during territory establishment in blackbirds. Anim Behav 64:207–213

Buchholz R (1997) Male dominance and variation in fleshy head ornamentation in wild turkeys. J Avian Biol 28:223–230

Burkhardt D (1989) UV vision: a bird's eye view of feathers. J Comp Physiol A 164:787–796

Burley N, Price DK, Zann RA (1992) Bill color, reproduction and condition effects in wild and domesticated zebra Finches. Auk 109:13–23

Cramp S, Simmons KEL (1980) The birds of the western Palearctic, vol 2. Oxford University Press, Oxford

Cuthill IC, Partridge JC, Bennett ATD, Church SC, Hart NS, Hunt S (2000) Ultraviolet vision in birds. Adv Study Behav 29:159–214

Darlington RB, Smulders TV (2001) Problems with residual analysis. Anim Behav 62:599–602

Delahay RJ, Speakman JR, Moss R (1995) The energetic consequences of parasitism—Effects of a developing infection of *Trichostrongylus tenuis* (Nematoda) on red grouse (*Lagopus*

lagopus scoticus): energy balance, body weight and condition. Parasitology 110:473–482
Dresp B, Jouventin P, Langley K (2005) Ultraviolet reflecting photonic microstructures in the king penguin beak. Biol Lett 1:310–313
Egeland ES, Parker H, Liaaenjensen S (1993) Carotenoids in combs of capercaillie (*Tetrao urogallus*) fed defined diets. Poultry Sci 72:747–751
Endler JA (1990) On the measurement and classification of colour in studies of animal colour patterns. Biol J Linn Soc 41:315–352
Faivre B, Gregoire A, Preault M, Cezilly F, Sorci G (2003) Immune activation rapidly mirrored in a secondary sexual trait. Science 300:103
García-Berthou E (2001) On the misuse of residuals in ecology: testing regression residuals vs. The analysis of covariance. J Anim Ecol 70:708–711
Hamilton WD, Zuk M (1982) Heritable true fitness and bright birds—a role for parasites. Science 218:384–387
Hausmann F, Arnold KE, Marshall NJ, Owens IPF (2003) Ultraviolet signals in birds are special. Proc R Soc Lond B 270:61–67
Hill GE (2000) Energetic constraints on expression of carotenoid-based plumage coloration. J Avian Biol 31:559–566
Hill GE (2002) A red bird in a brown bag: the function and evolution of ornamental plumage coloration in the house finch. Oxford University Press, Oxford
Hill GE, Brawner WR (1998) Melanin-based plumage coloration in the house finch is unaffected by coccidial infection. Proc R Soc Lond B 265:1105–1109
Hill GE, Inouye CY, Montgomerie R (2002) Dietary carotenoids predict plumage coloration in wild house finches. Proc R Soc Lond B 269:1119–1124
Hollett KG, Thomas VG, MacDonald SD (1984) Structural and functional aspects of supraorbital combs of grouse. In: Hudson P, Lovel TWI (eds) Third International Grouse Symposium. World Pheasant Association, York, pp 193–211
Hudson PJ (1986) The red grouse: the biology and management of a wild gamebird. The Game Conservancy Trust, Fordingbridge
Hunt S, Cuthill IC, Bennett ATD, Griffiths R (1999) Preferences for ultraviolet partners in the blue tit. Anim Behav 58:809–815
Hunt S, Cuthill IC, Bennett ATD, Church SC, Partridge JC (2001) Is the ultraviolet waveband a special communication channel in avian mate choice? J Exp Biol 204:2499–2507
Hunt S, Kilner RM, Langmore NE, Bennett ATD (2003) Conspicuous, ultraviolet-rich mouth colours in begging chicks. Proc R Soc Lond B 270:S25–S28
Johnsen A, Andersson S, Ornborg J, Lifjeld JT (1998) Ultraviolet plumage ornamentation affects social mate choice and sperm competition in bluethroats (Aves: *Luscinia s. svecica*): a field experiment. Proc R Soc Lond B 265:1313–1318
Johnsgard PA (1983) The grouse of the world. Croom Helm, Beckenham, UK
McGraw KJ, Ardia DR (2003) Carotenoids, immunocompetence, and the information content of sexual colors: an experimental test. Am Nat 162:704–712
McGraw KJ, Hudon J, Hill GE, Parker RS (2005a) A simple and inexpensive chemical test for behavioral ecologists to determine the presence of carotenoid pigments in animal tissues. Behav Ecol Sociobiol 57:391–397
McGraw KJ, Hill GE, Parker RS (2005b) The physiological costs of being colourful: nutritional control of carotenoid utilization in the American goldfinch, *Carduelis tristis*. Anim Behav 69:653–660
McGraw KJ, Correa SM, Adkins-Regan E (2006) Testosterone upregulates lipoprotein status to control sexual attractiveness in a colorful songbird. Behav Ecol Sociobiol 60:117–122
Mínguez-Mosquera I (1993) Clorofilas y carotenoides en tecnologia de alimentos. Universidad de Sevilla, Sevilla, Spain
Møller AP, Biard C, Blount JD, Houston DC, Ninni P, Saino N, Surai PF (2000) Carotenoid-dependent signals: Indicators of foraging efficiency, immunocompetence or detoxification ability? Avian Poult Biol Rev 11:137–159
Moss R (1972) Food selection by red grouse (*Lagopus lagopus scoticus* (Lath.)) in relation to chemical composition. J Anim Ecol 41:411–423
Mougeot F, Arroyo BE (2006) Ultraviolet reflectance by the cere of raptors. Biol Lett 2:173–176
Mougeot F, Redpath S (2004) Sexual ornamentation relates to immune function in male red grouse *Lagopus lagopus scoticus*. J Avian Biol 35:425–433
Mougeot F, Irvine J, Seivwright LJ, Redpath S, Piertney SB (2004) Testosterone, immunocompetence and honest sexual signaling in male red grouse. Behav Ecol 15:630–637
Mougeot F, Redpath SM, Leckie F (2005a) Ultra-violet reflectance of male and female red grouse, *Lagopus lagopus scoticus*: sexual ornaments reflects nematode parasite intensity. J Avian Biol 36:203–209
Mougeot F, Dawson A, Redpath S, Leckie F (2005b) Testosterone and autumn territorial behaviour in male red grouse *Lagopus lagopus scoticus*. Horm Behav 47:576–584
Mougeot F, Evans S, Redpath S (2005c) Interactions between population processes in a cyclic species: parasites reduce autumn territorial behaviour in male red grouse. Oecologia 144:289–298
Mougeot F, Redpath S, Piertney SB, Hudson PJ (2005d) Separating behavioural and physiological mechanisms in testosterone mediated trade-offs. Am Nat 166:158–168
Mundinger PC (1972) Annual testicular cycle and bill color change in the eastern American goldfinch. Auk 89:403–419
Negro JJ, Figuerola J, Garrido J, Green AJ (2001) Fat stores in birds: an overlooked sink for carotenoid pigments? Funct Ecol 15:297–303
Olson VA, Owens IPF (1998) Costly sexual signals: are carotenoids rare, risky or required? Trends Ecol Evol 13:510–514
Owens IPF, Short RV (1995) Hormonal basis of sexual dimorphism in Birds—Implications for new theories of sexual selection. Trends Ecol Evol 10:44–47
Peters A, Denk AG, Delhey AG, Kempenaers B (2004) Carotenoid-based bill colour as an indicator of immunocompetence and sperm performance in male mallards. J Evol Biol 17:1111–1120
Redpath S, Mougeot F, Leckie F, Evans S (2006) The effects of autumn testosterone on survival and productivity in red grouse *Lagopus lagopus scoticus*. Anim Behav 71:1297–1305
Rintamaki PT, Hoglund J, Karvonen E, Alatalo RV, Bjorklund N, Lundberg A, Ratti O, Vouti J (2000) Combs and sexual selection in black grouse (*Tetrao tetrix*). Behav Ecol 11:465–471
SAS (2001) SAS/STAT user's guide, version 8.01. SAS Institute, Cary, NC
Seivwright LJ, Redpath S, Mougeot F, Watt L, Hudson PJ (2004) Faecal egg counts provide a reliable measure of Trichostrongylus tenuis intensities in free-living red grouse *Lagopus lagopus scoticus*. J Helminthol 78:69–76
Seivwright LJ, Redpath S, Mougeot F, Leckie F, Hudson PJ (2005) Interactions between intrinsic and extrinsic mechanisms in a cyclic species: testosterone increases parasite infection in red grouse. Proc R Soc Lond B 272:1299–1304
Shawkey MD, Hill GE (2005) Carotenoids need structural colours to shine. Biol Lett 1:12–124
Siitari H, Viitala J (2002) Behavioural evidence for ultraviolet vision in a tetraonid species: foraging experiment with black grouse *Tetrao tetrix*. J Avian Biol 33:199–202
Stahl W, Sies H (2002) Carotenoids and protection against solar UV radiation. Skin Pharmacol Appl Skin Physiol 15:291–296

Thompson CW, Hillgarth N, Leu M, McClure HE (1997) High parasite load in house finches (*Carpodacus mexicanus*) is correlated with reduced expression of a sexually selected trait. Am Nat 149:270–294

von Schantz T, Bensch S, Grahn M, Hasselquist D, Wittzell H (1999) Good genes, oxidative stress and condition-dependent sexual signals. Proc R Soc Lond B 266:1–12

Wynn-Williams DD, Edwards HGM (2002) Environmental UV radiation: biological strategies for protection and avoidance. In: Horneck G, Baumstark-Khan C (eds) Astrobiology. The quest for the conditions of life. Springer, Berlin Heidelberg New York, pp 245–260

Zuk M, Johnsen TS, Maclarty T (1995) Endocrine-immune interactions, ornaments and mate choice in red jungle fowl. Proc R Soc Lond B 260:205–210