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The effects of preen oils and soiling on the UV–visible reflectance of carotenoid-pigmented feathers

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Abstract Plumage coloration, particularly when carotenoid-based, is important in social signaling in birds. Although feather color is a relatively stable trait, individuals may modify it with "cosmetic" substances such as preen oils. In addition, dirt accumulation may influence plumage coloration and further affect signal perception by receivers. Here, we analyze the separate potential effects of preen oils and soil accumulation on the reflectance properties of carotenoid-pigmented feathers across the visual range of most bird species, which includes the ultraviolet (UV). Using the yellow portion of tail feathers of Bohemian waxwings (*Bombycilla garrulus*), we performed two separate experiments where: (a) preen oils and/or soil were removed, or (b) preen oils (from black-billed magpies *Pica pica* or eagle owls *Bubo bubo*) were added. Preen oil addition reduced brightness but increased UV hue and yellow chroma. UV chroma was reduced by the addition of magpie (but not owl) preen oil. Soil accumulation had little effect on plumage reflectance in the UV range but significantly reduced yellow chroma. According to models of avian vision, both of these effects are detectable by birds and biologically meaningful when compared with natural variation between the sexes and age classes. We conclude that preen oil and soil accumulation can significantly affect the UV–visible reflectance of carotenoid-based plumages. As such traits typically advertise individual quality, preening and soiling have the potential to modify the information content of carotenoid-based plumage traits and how these signals are perceived by receivers.

Keywords Carotenoids · Coloration · Honest signals · Preen waxes · Uropygial gland · Sexual selection

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Introduction

Plumage coloration is a major target for sexual selection in birds (Hill and McGraw 2006a). Because it often reliably indicates individual quality, coloration can affect mating success or dominance, thereby influencing individual fitness (Hill and McGraw 2006a). Feather coloration is determined by differential wavelength reflection caused by feather microstructure, or by the selective wavelength absorption of pigments (e.g., melanins, carotenoids, porphyrins, etc.) (Hill and McGraw 2006b). Although plumage color may change slightly with time (e.g., Örnborg et al.

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2002; McGraw and Hill 2004; Figuerola and Senar 2005), the color of feathers is much more stable than that of other ornaments like beaks, legs, wattles, eye rings, or skin (e.g., Faivre et al. 2003; Martínez-Padilla et al. 2007; Pérez-Rodríguez 2008). However, there is increasing evidence that birds can modify feather reflectance by the application of some endogenous or exogenous agents such as oils, waxes, skin secretions, feather powders, and soil (Montgomerie 2006a; Delhey et al. 2007). The functions of these "cosmetic" modifications of plumage coloration are still poorly known, although some authors have highlighted their signaling potential, particularly in sexual selection (Negro et al. 1999; Delhey et al. 2007).

Preen oils are produced by the uropygial gland of most bird species and consist of a mixture of lipids that birds actively apply to feathers (Jacob and Ziswiler 1982). The primary role of uropygial gland secretions is to maintain the flexibility and waterproofing of feathers (Jacob and Ziswiler 1982), but recent studies have proposed other possible functions: preen oils could protect feathers from degrading bacteria (Burtt and Ichida 1999; Shawkey et al. 2003) or prevent feather color degradation due to exposure to sunlight (Reneerkens and Korsten 2004; Surmacki 2008). In addition, preen oils may modify plumage coloration by acting as optical filters (Piersma et al. 1999). Thus, birds could rapidly, and perhaps also reversibly, adjust plumage reflectance by applying variable amounts or types of preen oils to feathers (Reneerkens and Korsten 2004). Interestingly, preen oils mostly absorb light in the ultraviolet (UV) range (300–400 nm) of the spectrum (Reneerkens and Korsten 2004; Delhey et al. 2008). Many birds are sensitive to UV light (Cuthill 2006; see below) and respond to variation in plumage UV reflectance (Hausmann et al. 2003), so preen oil application could influence how UV-reflective plumages are perceived.

Plumage coloration can also be altered by the accumulation of soil and other exogenous substances (Montgomerie 2006a). In some cases, birds deliberately apply these substances for camouflage or status signaling (e.g., Montgomerie et al. 2001; Negro et al. 1999). However, in most cases, the accumulation of dirt in feathers is accidental and avoided by birds, which try to keep their plumage clean by the means of preening and bathing (Zampiga et al. 2004; Walther and Clayton 2005; Lenouvel et al. 2009). Dirt accumulation affects the insulating and waterproofing properties of the plumage, as well as their reflectance, which is often used as an indicator of individual quality (e.g., Zampiga et al. 2004).

Although some recent studies have tested the effect of preen oils on feather color, results have been mixed (Reneerkens and Korsten 2004; Surmacki and Nowakowski 2007; Delhey et al. 2008; López-Rull et al. 2010). Furthermore, besides the importance of including the UV range (300–400 nm) when studying avian coloration, this has been done by only two studies (Reneerkens and Korsten 2004; Delhey et al. 2008), none of which focused on carotenoid-based color. Carotenoid-pigmented plumages are particularly interesting because they are among the commonest signals of individual quality in birds (Hill and McGraw 2006a) and may reliably indicate foraging ability, parasite levels, and overall health status (reviewed by McGraw 2006). Moreover, carotenoid-pigmented feathers typically show a bimodal pattern of reflectance, with a marked peak in the UV and a plateau in the yellow–red visible spectrum (>500 nm) (e.g., Bleiweiss 2005; Shawkey and Hill 2005; Andersson and Prager 2006; see also Fig. 1). Therefore, preen oils and soil have the potential to influence reflectance in the UV and visible part of the spectrum and how these signals are perceived. Species differ in color perception, particularly in the UV part of the visual spectrum. In fact, birds can be split into two basic groups according to their visual system: species that are ultraviolet and violet sensitive, hereafter UVS and VS (Cuthill 2006). This is mainly based on the specific sensitivity of the cones responding to shorter wavelengths (UVS species possess higher sensitivity at shorter wavelengths than VS species), although there may be other differences in the rest of the visual spectrum. For instance, retinal oil droplets may modify color perception by birds due to their filtering properties (Vorobyev 2003; Hart and Hunt 2007).

Using two separate experiments, we tested the effects of preen oils and soiling on the UV–visible reflectance of the yellow band of tail feathers of Bohemian waxwings (*Bombycilla garrulus*). We selected this model species and trait because the yellow tail band is pigmented by carotenoids (primarily canary xanthophylls), as demonstrated by the closely related cedar waxwing *Bombycilla cedrorum* (Hudon and Brush 1989). This is a conspicuous color trait largely influenced by diet (Hudon and Brush 1989) that reflects both in the UV and visible parts of the spectrum (Fig. 1). Bohemian waxwings also possess yellow tips on primary feathers and red waxy tips on secondary feathers, also pigmented by carotenoids. In this study, we first test the effect of removal of preen oil and soil on the UV–visible reflectance of this trait. Also, in a second experiment, we test the effect of preen oil addition on the same trait. Although other closely related oscine species possess UVS cones (Ödeen and Håstad 2003), the visual range of waxwings is unknown. Therefore, we analyzed whether our experimental manipulations resulted in changes detectable by both UVS and VS species, in order to discuss the potential influence of these substances on the signal content of carotenoid-based plumage.

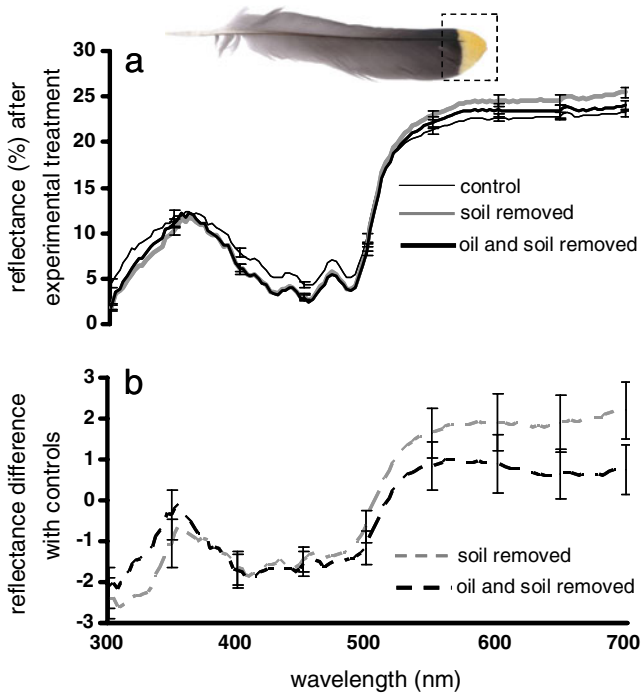

Fig. 1 Effects of experimental treatment in experiment 1 (see Methods) on feather reflectance. **a** Represents the reflectance spectra of the yellow tip of tail feathers of Bohemian waxwings (see photo insert) after soil removal (*gray solid line*), oil and soil removal (*thick black solid line*), and control treatment (*thin black solid line*). **b** Shows the difference in reflectance between feathers after soil (*gray dashed line*) or oil and soil removal (*black dashed line*) and controls. *Vertical bars* denote SE at 50-nm intervals

Methods

Feather collection

The birds used in this study were all carcasses salvaged after hitting windows on the campus of the University of Saskatchewan, Saskatoon, Canada. Bohemian waxwings are regular wintering birds in the city and can be commonly found in flocks of hundreds to about 2,000 (Mountjoy 2005 and personal observation). Multiple birds from a single flock often died together. On three occasions, five or six birds were seen striking a window at one time after it appeared that the flock had been disturbed by a predator.

Experiment 1: soil and preen oil removal

For this experiment, three adjacent tail feathers from each bird (N=40) were collected. We excluded the central and outer feathers to keep the sample from each bird homogeneous in terms of size, preen oil, and dirt content. Feathers from each individual were assigned to three experimental treatments: control, soil-removed, or preen oil- and soil-removed. All feathers were first dried in an oven at 40°C for 1 h. Feathers were then weighed (to the nearest 0.0001 g) using an electronic balance (Gibertini E-50-S, Milan, Italy), and the reflectance spectrum of the yellow tip (Fig. 1) was measured (see below) before the experimental manipulation. Preen oil and soil removal was performed using a similar protocol to that described by Surmacki and Nowakowski (2007). Oil/soil-removed feathers were put in a flask containing 50 ml of chloroform/methanol mixture (2:1), shaken, left for ca. 2 min, and shaken again before removing them from the flask and drying them in an oven at constant temperature (40°C). Soil-removed feathers were treated in the same way, but in this case, the flask contained 50 ml of distilled water. This treatment did not remove all the soil attached to the feather, as some of the dirt was mixed with preen oil (water is a polar solvent and was unable to remove dirt mixed with preen oil). Control feathers were shaken in an empty flask and placed in the same oven afterwards. Once they were completely dry after 5 h, feathers were weighed again and their reflectance spectra measured. These two measures of feather weight would allow us to confirm the effectiveness of our experimental manipulation, as feather weight changes were expected to follow the following pattern: oil/soil-removed feathers > soil-removed feathers > control feathers. During these procedures, feathers were carefully manipulated using gloves and forceps, and placed in hermetic plastic bags in between measurements.

Experiment 2: preen oil addition

For this experiment, 23 pairs of adjacent tail feathers were collected (as in experiment 1, outer and central feathers were excluded) and their preen oils and soil removed following the same procedure (i.e., chloroform/methanol washing) described above. After this, feathers were weighed and their color measured. We were not able to collect preen oils from waxwings, so we sampled uropygial gland secretions from two other species: black-billed magpies (*Pica pica*) and eagle owls (*Bubo bubo*) (three individuals from each species were sampled). Magpies were obtained from another study that required their euthanization and were kept frozen for 6 months. Magpie samples were obtained after defrosting the birds and gently pressing their uropygial glands the same day of the experiment. Eagle owl samples were collected from three live birds kept in a local rehabilitation center (Centro de Recuperación de Fauna Silvestre “El Chaparrillo”, Ciudad Real, Spain). These birds had stabilized bone fractures in the wings and could not be released to the wild but were in good condition as indicated by regular checks by veterinary staff of the center. Owl samples were collected in a similar way, and samples were kept frozen for 1 week until the

experiment was performed. We assumed that this short period of freezing does not produce substantial change in the optical properties of preen oils, as supported by recent evidence (Delhey et al. 2008).

Pairs of feathers from individual waxwings were assigned to two different groups depending on the origin (magpie or owl) of the preen oil employed. In the magpie group, each pair of feathers (N=13) was split into two groups: magpie preen oil-added ("magpie oil") or control ("magpie control"), i.e. no preen oil addition. Similarly, pairs of feathers assigned to the owl group (N=10) were split into owl preen oil-added ("owl oil") or control ("owl control"). Preen oils were defrosted at ambient temperature before application. For preen oil additions, a small drop of oil was applied at the base of the yellow tail band and carefully spread over the whole yellow distal part to be measured using a piece of plastic film. Control feathers were manipulated in the same way, but no substance was added. After these procedures, feathers were weighed again and their reflectance measured. These measures of feather weight before and after the addition of preen oil would allow us to verify that the additions were within the natural range (i.e., changes in feather weight in experiment 2 [oil addition] were similar to those of experiment 1 [oil removal]).

Color measurements

The reflectance of the yellow distal part of the tail feathers was measured using an AvaSpec USB2000 spectrophotometer connected to a deuterium–halogen light source (DH2000, Avantes, Eerbek, Netherlands) through a bifurcated fiber-optic probe providing a 45° to normal angle of illumination/recording. Three consecutive measurements were conducted per feather, removing the probe from the feather between measurements. Measurements were done in a partially dark room to avoid possible interference from ambient light. Reflectance values (in 3-nm steps, from 300 to 700 nm) were calculated relative to a Spectralon® 99% white standard reference (Labsphere, Congleton) and to the dark, and computed using the program Spectrawin 5.0. The white reference was checked every six feathers to ensure the stability of the light source.

Following Montgomerie (2006b) and Andersson and Prager (2006), we summarized spectral data by calculating the following variables: (1) mean brightness (average reflectance in the interval 300–700 nm), (2) UV hue (spectral location, in nanometers, of the reflectance peak in the UV range), (3) UV chroma (difference between maximum and minimum reflectance in the interval 300–400 nm, divided by brightness); (4) yellow hue (wavelength of maximum slope in the 400–700 interval), and (5) yellow chroma (difference between maximum and minimum reflectance in the interval 400–700 nm, divided by brightness). Correlations between these variables are shown in Online Resource 1.

Significant effects of our treatments on the color variables do not necessarily imply that these effects could be perceived by birds. Therefore, we also quantified whether spectral differences could be detectable by birds by using a color discrimination model (Vorobyev and Osorio 1998; Vorobyev et al. 1998). We used visual parameters of UVS (Hart et al. 2000) and VS birds (Hart 2002) to compute the chromatic (ΔS) and achromatic (ΔQ) contrasts in the avian visual space as a result of experimental manipulations. The unit for ΔS and ΔQ is the "just noticeable difference" (JND); values of ΔS or ΔQ higher than 1 indicate color differences noticeable by birds. ΔS and ΔQ calculations were done using the program Avicol (Gomez 2006). Further mathematical details of these models can be found in Delhey et al. (2008), Lenouvel et al. (2009), and Avilés and Soler (2009).

Statistics

We tested the effect of treatments on color variables using general linear mixed models. In experiment 1, sampling time ("before" or "after" experimental manipulation) and treatment (control, soil-removed, or oil/soil-removed) were entered as fixed effects and individual feather as a random variable to account for repeated measures (before and after treatment) on the same feather. In experiment 2, the statistical models included time, treatment (preen oil-added or control), and oil type (owl or magpie) as fixed factors. To assess whether the effect of preen oils differed depending on preen oil type at different times, the three-way interaction of time × treatment × oil type and all two-way interactions between these effects were also included in the model.

To assess whether changes in feather reflectance in both experiments would be noticeable by birds, we computed ΔS and ΔQ (for UV and UVS eye types) between control and experimental feathers before and after manipulations in both experiments. We tested whether each contrast between control and treated feathers significantly increased as a result of the treatments using paired t tests.

Mean brightness, ΔS, and ΔQ values were log-transformed to achieve normality (Kolmogorov–Smirnov test), although untransformed values were used in tables and graphs. All tests are two tailed, and all data are given as mean ± standard error (SE).

Results

Experiment 1: soil and preen oil removal

Experimental treatments caused significant differences in the percentage of weight lost by feathers (time × treatment

interaction: $F_{2,117}$=48.5, P<0.001). Oil/soil-removed feathers lost more weight than soil-removed feathers and soil-removed feathers lost more weight than control feathers, and all differences between groups were significant (all P< 0.001; Fig. 2a).

The effect of experimental treatment on feather color is shown in Tables 1 and 2 and Figs. 1 and 2. Mean brightness was not affected by treatment. In contrast, UV hue and UV chroma showed significant variation depending on the treatment (Fig. 2c, d). Although oil/soil-removed feathers did not show a significant change in UV hue ($F_{1,39}$=2.42, P=0.13), variations exhibited were significantly different from those of both control feathers (time × treatment interaction: $F_{1,78}$=5.00, P=0.03) and soil-removed feathers (time × treatment interaction: $F_{1,78}$=11.1, P=0.001). In soil–removed feathers, UV hue increased over time ($F_{1,39}$=11.3, P=0.002). In contrast, UV hue did not increase significantly over time in control feathers ($F_{1,39}$=2.53, P=0.12). Overall, the increase in UV hue observed in soil-removed feathers was not statistically different from that observed in control feathers (sampling time × treatment interaction: $F_{1,78}$=0.70, P=0.41).

UV chroma did not change significantly in control and soil-removed feathers (both P>0.15), the changes being similar among treatment groups (time × treatment interaction: $F_{1,78}$=1.06, P=0.31). However, oil/soil-removed feathers showed a significant increase in UV chroma ($F_{1,39}$=37.3, P<0.001), different from that of control ($F_{1,78}$=10.6, P= 0.002) and soil-removed feathers ($F_{1,78}$=16.1, P<0.001) (Fig. 2d).

Yellow hue increased similarly in all groups (no significant time × treatment interaction, Table 1 and Fig. 2e). Changes in yellow chroma differed, although not significantly, between treatments (Table 1, Fig. 2f). Control and oil/soil-removed feathers showed no significant change in yellow chroma ($F_{1,39}$=0.03, P=0.87 and $F_{1,39}$=2.03, P=0.16, respectively). In contrast, soil-removed feathers showed a significant increase in yellow chroma ($F_{1,39}$=12.7, P<0.001). Hence,

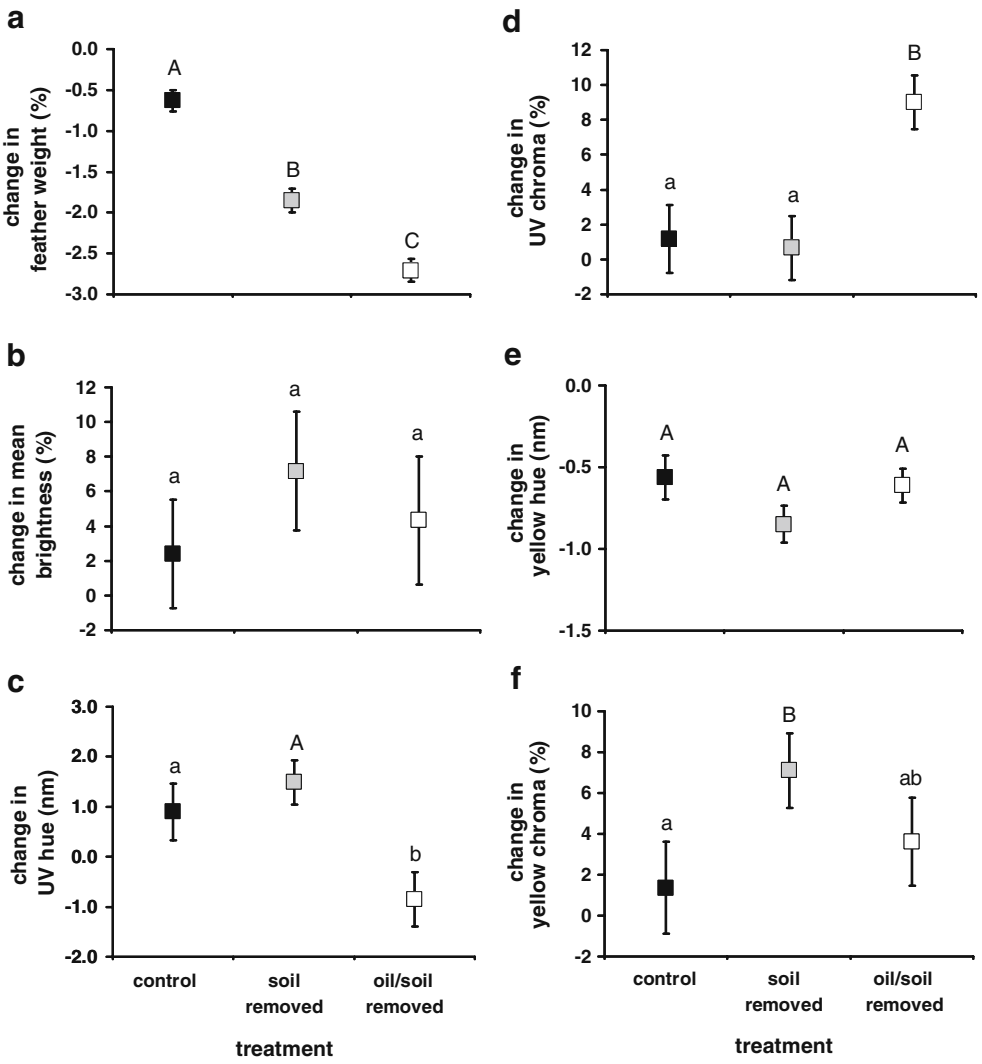

Fig. 2 Changes, in mean ± SE, according to treatment within experiment 1, in **a** feather weight (in percent), **b** mean brightness (in percent), **c** UV hue (in nanometers), **d** UV chroma (in percent), **e** yellow hue (in nanometers), and **f** yellow chroma (in percent). *Black, gray, and open squares* correspond to control, soil-removed, and soil- and preen oil-removed feathers, respectively. *Different letters above symbols* indicate significant (P<0.05) differences between treatment groups. *Capital letters* indicate a change significantly different from zero; *uncapitalized letters* indicate a change not significantly different from zero (non-significant change)

Table 1 Effect of treatments (experiment 1; see Methods) on feather brightness, UV hue, UV chroma, yellow hue, and yellow chroma of the carotenoid-pigmented portion of waxwing tail feathers

	Brightness			UV hue			UV chroma			Yellow hue			Yellow chroma		
	F	*df*	*P*	*F*	*df*	*P*	*F*	*df*	*P*	*F*	*df*	*P*	*F*	*df*	*P*
Time (*T*)	1.24	1, 117	0.267	2.94	1, 117	0.089	16.3	1, 117	<0.001	98.5	1, 117	<0.001	8.45	1, 117	0.004
Treatment (TR)	0.77	2, 117	0.464	0.56	2, 117	0.571	3.21	2, 117	0.043	0.25	2, 117	0.780	7.58	2, 117	<0.001
T × TR	0.48	2, 117	0.621	5.48	2, 117	0.005	9.19	2, 117	<0.001	1.70	2, 117	0.187	2.42	2, 117	0.093

Time (before or after) and treatment (control, soil-removed, and soil/oil-removed) were included as fixed effects. "Individual feather" was entered as a random variable

changes in yellow chroma differed significantly between control and soil-removed feathers ($F_{1,78}$=5.62, P=0.02), but not between control and oil/soil-removed feathers ($F_{1,78}$= 0.98, P=0.32) or between soil-removed and oil/soil-removed feathers ($F_{1,78}$=1.28, P=0.26).

All these effects on feather reflectance parameters resulted in changes in chromatic distances (ΔS) noticeable by UVS or UV visual systems (Table 2), although changes were closer to the discrimination threshold in the later. In contrast, no detectable changes in the achromatic distance (ΔQ) were found between treatments, regardless of the visual system (UVS or VS; Table 2).

Experiment 2: preen oil addition

Preen oil addition caused an increase in feather weight in oiled feathers (time × treatment: $F_{1,42}$=53.0, P<0.001) which was similar in magpie-oil and owl-oil feathers (time × treatment × type of preen oil interaction: $F_{1,\ 42}$=0.07, P=0.79) (Fig. 3a). The magnitude of this increase (2.0±0.35%) was similar to the net decrease in feather weight of oil/soil removal (i.e., after discounting the change of control and soil-removed feathers (1.6±0.9%), and the differences between the two were non-significant ($F_{1,61}$=2.06, P=0.15). This indicated that the amount of preen oil added was within the natural range found in waxwing tail feathers.

The effects of experimental preen oil additions on feather color variables are shown in Table 3 and Figs. 3 and 4. Mean brightness decreased in control feathers, but this was significant only in owl group (magpie control: $F_{1,\ 12}$=1.99, P=0.18; owl control: $F_{1,\ 9}$=7.62, P=0.02), although this decrease was much more pronounced as a result of preen oil addition (significant time × treatment interaction), irrespective of the type of oil (Table 3, Fig. 4b). However, these changes in feather brightness may not be noticeable by birds, as shown by the effects on achromatic contrast using UVS (magpie oil: t_{12}=1.85, P= 0.09; owl oil: t_9=0.01, P=0.99) or VS visual systems (magpie oil: t_{12}=1.89, P=0.08; owl oil: t_9=0.16, P=0.87) (Table 2).

UV hue increased after preen oil addition (magpie-oil feathers: $F_{1,12}$=11.1, P=0.006; owl-oil feathers: $F_{1,\ 9}$=81.0, P<0.001), the effect being similar for both types of preen oil (non-significant time × treatment × oil type interaction; Table 3, Figs. 3 and 4c). In contrast, control feathers showed no significant changes in UV hue during the experiment (magpie control: $F_{1,12}$=3.1, P=0.1; owl control: $F_{1,9}$=198, P=0.19).

Table 2 Absolute change in chromatic (ΔS) and achromatic (ΔQ) distances (in JNDs) between control and experimental feathers during experiment 1 (soil/preen oil removal) and experiment 2 (magpie or eagle owl preen oil addition)

		UVS eyes		VS eyes	
		ΔS	ΔQ	ΔS	ΔQ
Experiment 1	Control vs. soil-removed	2.1±0.94*	−0.5±0.56	1.7±0.83*	−0.18±0.54
	Control vs. oil/soil-removed	2.4±1.1*	−0.4±0.57	1.8±0.94**	−0.2±0.53
	Soil removed vs. oil/soil-removed	1.5±0.44*	0.20±0.40	1.15±0.40**	0.22±0.40
Experiment 2	Control vs. magpie oil addition	3.4±1.1*	1.4±0.76***	4.1±1.24*	1.5±0.80***
	Control vs. owl oil addition	3.1±1.7**	0.59±0.92	2.4±1.5**	0.48±0.87

Values correspond to post-treatment contrast minus pre-treatment contrasts between the specified groups, calculated for UVS and VS eyes. For experiment 1, the change in ΔS and ΔQ between "soil-removed" and "oil/soil-removed" feathers is also shown. Levels of significance of the changes after paired *t* tests (pre-treatment contrasts vs. post-treatment contrasts between the specified groups) are noted by asterisks (*P<0.01; **P<0.05; ***P<0.1). See Methods for further details

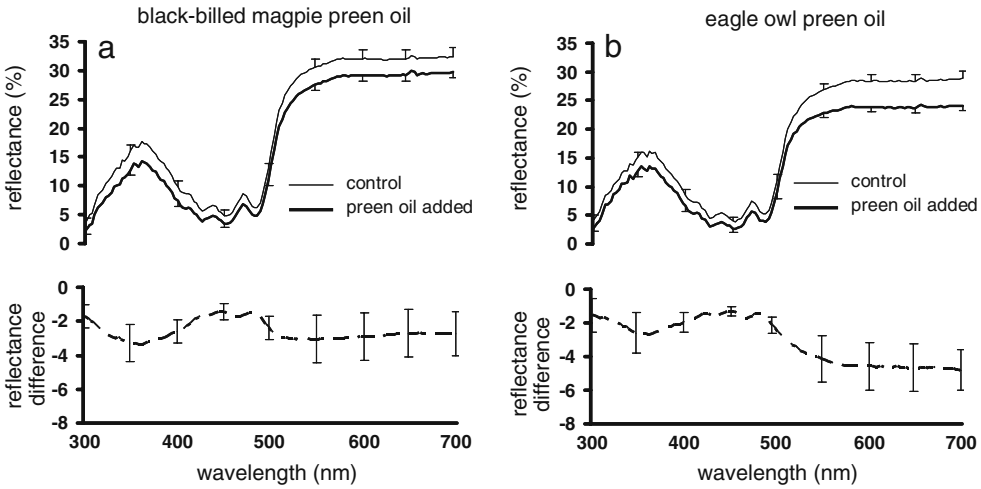

Fig. 3 Effects of **a** black-billed magpie and **b** eagle owl preen oil addition on waxwing feather reflectance. *Thin lines* represent intact (control) feathers, *thick lines* represent feathers with preen oil applied. *Dashed lines in the lower graphs* represent the difference in reflectance between control and feathers with magpie or owl preen oil. *Vertical bars* denote SE at 50-nm intervals

UV chroma was also affected by the experimental treatment (Fig. 4d); however, in this case, the effect depended on the type of preen oil applied (significant time × treatment × type of preen oil interaction, Table 3). Magpie control and magpie-oil feathers showed a decrease in UV chroma ($F_{1,\ 12}$=5.84, P=0.03 and $F_{1,\ 12}$=20.8, P<0.001, respectively), which was significantly more pronounced in the magpie control than in magpie-oil feathers (time × treatment interaction: $F_{1,\ 24}$=8.36, P=0.008) (Fig. 3a). In contrast, owl control and owl-oil feathers did not show any change in UV chroma (time: $F_{1,\ 18}$=0.78, P=0.39; time × treatment interaction: $F_{1,\ 18}$= 0.39, P=0.54) (Fig. 3b).

Yellow hue decreased during the experiment, similarly so in the different treatment groups (Table 3, Fig. 4e). Finally, yellow chroma increased in all groups, although this increase was significantly greater in feathers where preen oils were added, irrespective of the type of oil employed (Table 3, Fig. 4f).

All these changes in feather reflectance resulted in a significant increase in chromatic distance between control and experimental feathers for species possessing UVS (magpie oil: t_{12}=3.68, P=0.003; owl oil: t_9=2.73, P= 0.02) or VS visual systems (magpie oil: t_{12}=3.90, P=0.002; owl oil: t_9=2.44, P=0.03) (Table 2).

Discussion

In this study, we tested the effect of preen oils and soil on feather reflectance. This is the first experimental attempt to tease apart the effects of these two factors on feather reflectance. Also, this is the first study analyzing the effects of preen oils on the reflectance of a carotenoid-based plumage across the entire visual range of most birds (300–700 nm) and taking into account the color discriminability of the avian visual system.

Preen oil addition increased UV hue (feather reflectance peaked at longer wavelengths in the 300–400 nm interval), and in the case of magpie's uropygial secretions, it also reduced UV chroma. This is consistent with results of

Table 3 Effect of treatments (experiment 2; see Methods) on feather brightness, UV hue, UV chroma, yellow hue, and yellow chroma of the carotenoid-pigmented portion of waxwing tail feathers

	Brightness			UV hue			UV chroma			Yellow hue			Yellow chroma		
	F	*df*	*P*	*F*	*df*	*P*	*F*	*df*	*P*	*F*	*df*	*P*	*F*	*df*	*P*
Time (*T*)	75.6	1, 42	<0.001	42.0	1, 42	<0.001	17.5	1, 42	<0.001	36.1	1, 42	<0.001	141.0	1, 42	<0.001
Oil type (OT)	6.14	1, 42	0.02	2.89	1, 42	0.09	2.59	1, 42	0.12	2.41	1, 42	0.13	0.00	1, 42	0.98
Treatment (TR)	4.21	1, 42	0.04	0.41	1, 42	0.53	0.48	1, 42	0.49	0.05	1, 42	0.83	0.83	1, 42	0.37
T × OT	4.92	1, 42	0.03	1.13	1, 42	0.29	8.61	1, 42	0.005	0.77	1, 42	0.39	0.02	1, 42	0.90
T × TR	14.3	1, 42	<0.001	11.7	1, 42	0.001	2.40	1, 42	0.13	2.58	1, 42	0.12	8.86	1, 42	0.005
TR × OT	0.39	1, 42	0.53	0.11	1, 42	0.74	0.26	1, 42	0.61	0.02	1, 42	0.88	0.10	1, 42	0.76
T × TR × OT	0.05	1, 42	0.82	1.22	1, 42	0.28	5.90	1, 42	0.02	0.01	1, 42	0.90	0.32	1, 42	0.57

Time (before or after) and treatment (control or preen oil-added) and the type of preen oil (magpie or owl) were included as fixed effects. "Individual feather" was entered as a random variable

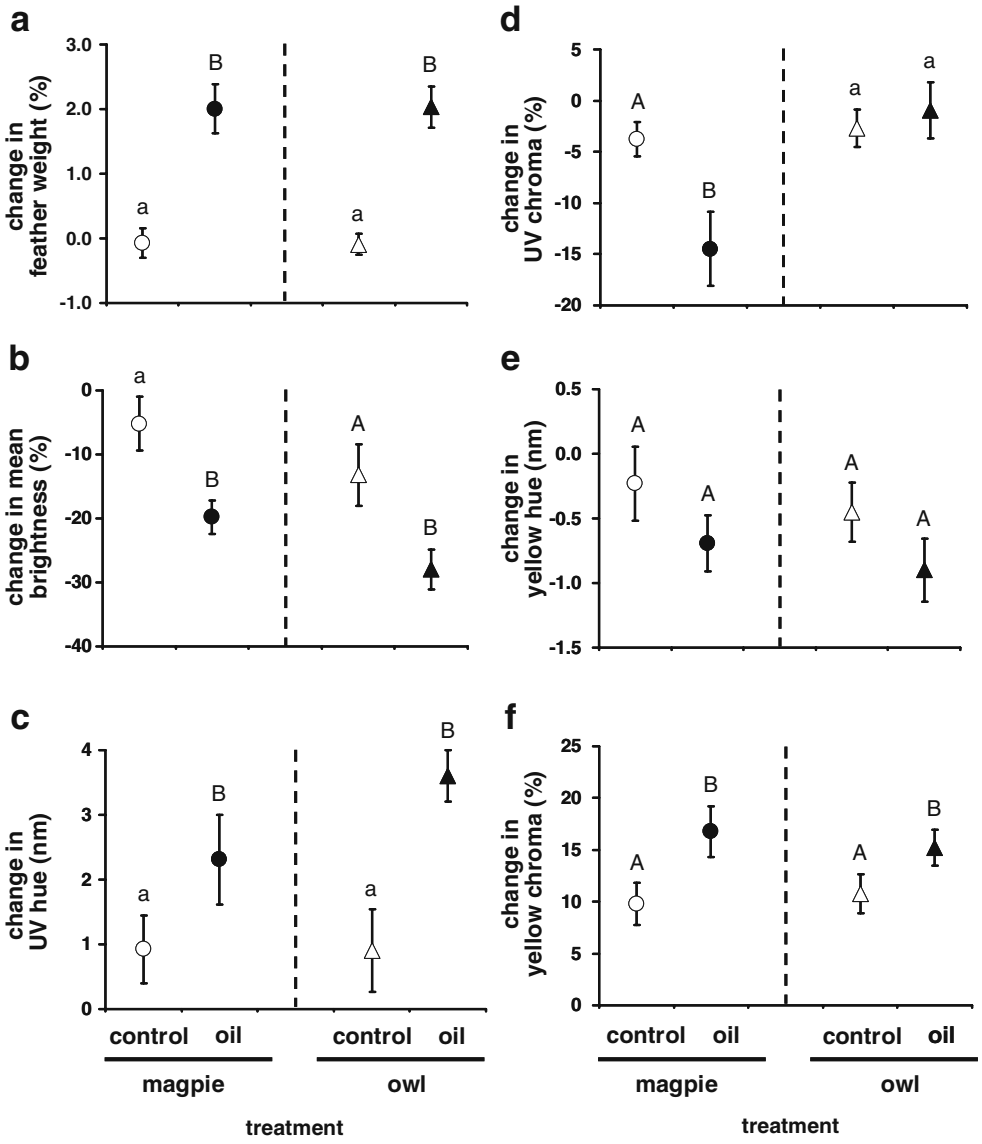

Fig. 4 Changes, in mean ± SE, according to treatment within experiment 2, in **a** feather weight (in percent), **b** mean brightness (in percent), **c** UV hue (in nanometers), **d** UV chroma (in percent), **e** yellow hue (in nanometers), and **f** yellow chroma (in percent) according to treatment within experiment 2. *Circles and triangles* correspond to the magpie and owl preen oil group, respectively, whereas *open and black symbols* correspond to control and oiled feathers, respectively. *Different letters above symbols* indicate significant ($P<0.05$) differences between treatment groups. *Capital letters* indicate a change significantly different from zero; *uncapitalized letters* indicate a change not significantly different from zero (non-significant change)

Delhey et al. (2008), showing a decrease in UV chroma and overall brightness when preen oils were applied to white feathers of mallards (*Anas platyrynchos*) or UV-reflective feathers of blue tits (*Parus caeruleus*). In contrast, Reneerkens and Korsten (2004) did not find any effect of preen oil removal on the reflectance of feathers of red knots (*Calidris canutus*), possibly because these feathers were pigmented by melanins and reflected little in the UV range. The reduction of UV reflectance by preen oils is likely explained by the relatively higher absorbance of preen oils at short wavelengths (Reneerkens and Korsten 2004; Delhey et al. 2008).

Preen oil addition also increased yellow chroma but did not affect yellow hue. This is consistent with López-Rull et al. (2010), who reported an increase in yellow/red chroma after preen oil addition to carotenoid-pigmented feathers of house finches (*Carpodacus mexicanus*). In contrast, Surmacki and Nowakowski (2007) reported a somewhat different result, as yellow hue, but not yellow chroma, was affected by preen oil removal. However, that study used a different method to measure color, based on digital photographs, which may explain, in part, these discrepancies. In addition, their experimental treatment also removed all possible dirt attached to the feather surface, making it difficult to distinguish the effect of preen oil vs. soiling. We also found that preen oil addition reduced mean brightness, consistent with the results of Surmacki and Nowakowski (2007) and López-Rull et al. (2010), although these effects were below the discrimination threshold of birds. A positive effect of preen oil accumulation on feather glossiness might have also been expected (Delhey et al. 2007). However, unfortunately, glossiness could not be quantified in our study from the reflectance spectra measured.

Given the importance of plumage coloration for social signaling (Hill and McGraw 2006a), the effects of preen oils and dirt on feather coloration could influence how carotenoid-based signals of individual quality are perceived. In fact, our experimental treatments influenced color traits that differed between the sexes and age classes of Bohemian waxwings (see Online Resource 2), and these effects were noticeable either by the UVS or VS species models, as revealed by the significant changes in chromatic contrasts between control and experimental feathers. Thus, it seems reasonable to expect that soil and preen oil accumulation would affect the signal content of carotenoid-based coloration in at least this species.

The yellow chroma of carotenoid-pigmented feathers reflects their carotenoid content (Saks et al. 2003; Isaksson et al. 2008), which may be informative of several aspects of individual quality (reviewed by McGraw 2006). Therefore, the positive effect of preen oils on yellow chroma found in this study suggests that they could serve a cosmetic function (Delhey et al. 2007), acting as enhancers of the signal. The honesty of this signal-enhancing mechanism would require the existence of some costs for preen oil application (Zahavi and Zahavi 1997). These costs may arise directly from preen oil production: the activity of the uropygial gland is stimulated by increased testosterone (Ghosh and Bhattacharyya 1996) and maintaining high testosterone levels may be costly due to the collateral immunosuppressive effects of the hormone (Folstad and Karter 1992). Also, the preen oil production may be constrained by individual nutritional status (Oka and Okuyama 2000). Preen oil application to feathers may also be influenced by indirect costs, such as the amount of time and energy required for preening. This is not a negligible cost, as preening activities consume a significant portion of a bird's daytime budget (Walther and Clayton 2005), and the energetic cost of preening has been estimated as twice the basal metabolic rate (Goldstein 1988).

We also found that preen oil addition increased UV hue and, in the case of magpie preen oil, UV chroma. Unlike yellow chroma, the UV reflectance of carotenoid-pigmented plumages is caused by feather microstructures (Shawkey and Hill 2005). Although UV and yellow reflectance peaks of carotenoid-pigmented feathers have been positively related (Mays et al. 2004; Shawkey et al. 2006), UV chroma does not seem to be related to the feather's carotenoid content (Shawkey et al. 2006). Therefore, more research is needed on the biological meaning of UV reflectance of carotenoid-based plumages (e.g., Shawkey and Hill 2005; Shawkey et al. 2006; Galván et al. 2008) to interpret the effects of preen oils on UV reflectance. Nevertheless, the effect of preen oils on UV reflectance could be important in those species with structural plumage ornaments where high UV-blue reflectance plays a role in social signaling (e.g., Sheldon et al. 1999; Siefferman and Hill 2005). In those cases where high UV chroma/low UV hue are cues for sexual selection, individuals may experience a tradeoff between signal maximization and receiving the benefits of a properly oiled plumage (Jacob and Ziswiler 1982; Burtt and Ichida 1999), thus adding an extra cost to signal maximization that may increase signal honesty.

Another interesting finding is that preen oil from black-billed magpies (Passeriformes) affected UV chroma, whereas preen oils from eagle owls (Strigiformes) did not. A recent study (Delhey et al. 2008) tested the effect of preen oils from 51 species belonging to 12 avian orders on UV/visible reflectance of a white–UV-reflective surface (Teflon™ tape) and found that uropygial secretions reduced overall brightness and especially UV chroma, and particularly for non-passerine secretions. Unfortunately, that study did not include preen oils from any species of owl. The contrasting effect of magpie and owl preen oils on feather UV chroma is difficult to interpret under a signaling scenario because magpies and owls have different visual systems. Corvids, where black-billed magpies are included, are VS species (Ödeen and Håstad 2003) and can perceive variations in the near-UV range (Cuthill 2006). In contrast, owls and other nocturnal raptors apparently lack UVS or VS cones (Bowmaker and Martin 1978; Cuthill 2006), although a recent experiment (Parejo et al. 2010) indicates that at least some species can detect variations in the UV range. We were not able to use preen oils from waxwings; however, the removal of waxwing preen oil and magpie preen oil addition exerted, as expected, similar but opposite effects on feather reflectance parameters. We believe that our results are biologically meaningful given that the optical properties of uropygial secretions of all passeriforms are roughly similar (Delhey et al. 2008).

Our treatment with water (soil-removed feathers) did not totally remove soil, and a certain proportion of dirt likely remained attached to the feathers, mixed with preen oils (personal observation). Soil and preen oil removal reduced UV hue and increased UV chroma (which is basically opposite to the effect of preen oil addition), indicating that soil appears to have little effect on plumage reflectance in the UV range as compared to preen oil. However, soil and preen oil had opposite effects on yellow chroma: preen oil addition increased yellow chroma, and the same effect was obtained after partial soil removal. In agreement with this result, we found that concurrent removal of preen oil and soil partially reverted the effect of soil removal alone (Fig. 2f). As yellow chroma is often indicative of individual quality (see above), our results are consistent with the hypothesis that dirt accumulation on feathers could reduce plumage attractiveness, and so preening may enhance the attractiveness of

individuals (Zampiga et al. 2004; Montgomerie 2006a; Lenouvel et al. 2009; López-Rull et al. 2010).

We should mention that, unexpectedly, control feathers showed some changes in reflectance during the experiment (particularly in Experiment 2). This is likely to be attributable to feather handling (e.g., disordered feather barbs) or some minor degradation of pigments during the experiment. Although this does not affect our results as all feathers were manipulated in the same way, extreme care should be taken when handling feathers in future studies involving color measurements.

In conclusion, although plumage coloration has been traditionally considered a relatively static trait, several studies have shown that it can change due to feather wear or pigment degradation (Örnborg et al. 2002; McGraw and Hill 2004; Figuerola and Senar 2005). Our results suggest that feather coloration may also be modified by preen oil content and soiling of feathers. As plumage coloration usually acts as a cue for sexual selection, it is likely that individuals are able to modify their attractiveness by investing in preening activities, thereby enhancing signal expression.

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