Effects of Sunlight Exposure on Carotenoid-Based and Structural Coloration of the Blue-tailed Bee-eater - Efectos de la Exposición a la Luz Solar en la Coloración Basada en Carotenoides y Estructural de Merops philippinuse

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EFFECTS OF SUNLIGHT EXPOSURE ON CAROTENOID-BASED AND STRUCTURAL COLORATION OF THE BLUE-TAILED BEE-EATER

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Abstract. After a bird's molt is complete, the coloration of its plumage may be altered by external factors such as soiling. We tested how exposure to sunlight affects plumage coloration derived from carotenoid pigments and feather nanostructure. We studied these changes in the Blue-tailed Bee-eater (*Merops philippinus*) because that species combines yellow chin feathers (colored by carotenoid pigments), green back feathers (colored by a combination of carotenoid pigments and feather nanostructure), and turquoise-blue rump feathers (colored by feather nanostructure). We measured reflectance of feather samples and then exposed them to sunlight in boxes that allowed penetration of both UV and visible wavelengths of light. After exposure to sunlight, reflectance spectrometry revealed that all three regions became less colorful. According to models of avian sight, chromatic aspects of color change were too small to be perceived in the majority of individuals. In contrast, the reduction in brightness after exposure to sunlight was likely visible to birds in most cases. Moreover, our results suggest that types of feather coloration differ in sensitivity to sunlight-induced change in color that is visible to birds. Structural coloration appears to be the most resistant to sunlight, carotenoid-based coloration appears to be the most sensitive, and colors produced by a combination of carotenoid pigments and feather microstructure are intermediate in sensitivity. Overall, our study demonstrates that sunlight modifies plumage coloration between successive molts but the strength of this effect on the Blue-tailed Bee-eater is relatively small and depends on the mechanism of color production.

Key words: *bee-eaters*, *color bleaching*, *Coraciiformes*, *keratin*, Merops philippinus, *UV radiation*.

Efectos de la Exposición a la Luz Solar en la Coloración Basada en Carotenoides y Estructural de *Merops philippinuse*

Resumen. Luego que la muda de un ave se completa, la coloración de su plumaje puede alterarse por factores externos como teñirse o ensuciarse. Examinamos cómo la exposición a la luz solar afecta la coloración del plumaje derivada de pigmentos carotenoides y de la nanoestructura de las plumas. Estudiamos estos cambios en *Merops philippinus* porque esta especie combina plumas amarillas de la barbilla (coloreadas por pigmentos carotenoides), plumas verdes del lomo (coloreadas por una combinación de pigmentos carotenoides y la nanoestructura de las plumas) y plumas turquesa-azules de la rabadilla (coloreadas por pigmentos de melanina y por la nanoestructura de las plumas). Medimos la reflectancia de muestras de plumas y luego las expusimos a la luz solar en cajas que permitían la entrada de luz de longitudes de onda UV y visibles. Después de la exposición a la luz solar, la espectrometría de reflectancia reveló que las tres regiones se volvieron menos coloridas. Según modelos de visión de aves, los aspectos cromáticos del cambio de color fueron muy pequeños para ser percibidos por la mayoría de los individuos. En contraste, la reducción del brillo después de la exposición a la luz solar probablemente fue visible para la mayoría de las aves. Además, nuestros resultados sugieren que los tipos de coloración de las plumas difieren en su sensibilidad al cambio de color inducido por la luz solar que es visible para las aves. La coloración estructural parece ser la más resistente a la luz solar, la coloración basada en carotenoides parece ser la más sensible y los colores producidos por una combinación de pigmentos carotenoides y la microestructura de la pluma son intermedios en cuanto a su sensibilidad. Sobre todo, nuestro estudio demuestra que la luz solar modifica la coloración del plumaje pero que entre mudas consecutivas la fuerza de este efecto en *M. philippinus* es relativamente pequeña y depende del mecanismo de producción del color.

INTRODUCTION

Plumage coloration has long been recognized as a trait important in sexual selection, and many environmental factors influence feather coloration prior to or during molt (reviewed in Hill 2006). Recently, however, attention has focused on factors that affect plumage color after plumage development is complete, acknowledging that feather coloration is not necessarily a static trait. Such color changes can be influenced by keratophilic microbes (Shawkey et al. 2008), accumulation of soiling (Surmacki and Nowakowski 2007), preening

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behavior (Lenouvel et al. 2009), preen oil (Pérez-Rodríguez et al. 2011), abrasion (Willoughby et al. 2002), and exposure to sunlight (Surmacki 2008).

Plumage color is most commonly derived from pigments and/or feather microstructure (Hill and McGraw 2006). Carotenoid pigments are responsible for bright red, orange, and yellow colors (Hill and McGraw 2006). Noniridescent structural coloration results from the coherent reflection of light from nanostructural elements within the medullary layer of feather barbs and can appear blue, green, or many other colors (Prum 2006). Additionally, some green colors are produced by a combination of the structural blue component and yellow carotenoid pigments in the feather barbs (Prum 2006).

The destructive effect of solar radiation on feather coloration has been tested experimentally and quantitatively only once, and that study focused on the carotenoid-based yellow of the Great Tit (*Parus major*; Surmacki 2008). Feathers exposed to sunlight decreased in both saturation and brightness, and hues shifted toward shorter wavelengths of light, suggesting that the destruction of carotenoid pigments causes duller coloration (Surmacki 2008). Changes in saturation and hue were less pronounced in feathers that had been protected by UV screening, indicating that short-wave solar radiation is more destructive to carotenoid pigments than is long-wave solar radiation (Surmacki 2008). Correlative studies also suggest that sunlight may be responsible for seasonal changes in the carotenoid-based coloration of other species (e.g., McGraw and Hill 2004). Although colors produced by feather nanostructure change between molts (Örnborg et al. 2002, Delhey et al. 2006), the effects of solar radiation on structural coloration remain unstudied experimentally.

Here, we experimentally test the effect of sunlight on three types of plumage coloration in a single species, the Blue-tailed Bee-eater (*Merops philippinus*). Previous spectrometric studies of this species showed that different body regions are colored by carotenoid pigments, feather nanostructure, and a combination of both mechanisms (Siefferman et al. 2007). The reflectance spectrum of the yellow chin is typical of carotenoid xanthophylls (McGraw et al. 2001), while the turquoise-blue of the rump is likely a result of structural coloration alone (Silva et al. 2008). The noniridescent green of the back may derive from the combination of feather-barb nanostructure overlaid by carotenoid pigments (Prum 2006, Siefferman et al. 2007). Our goal was to characterize how exposure to sunlight changes the reflectance properties of different types of plumage coloration. We used two approaches. First, we compared common color variables (hue and chroma) calculated from reflectance curves obtained before and after exposure to sunlight. However, these variables should be treated as a potential but not necessarily as a perceived signal (Montgomerie 2006). Color perception of birds depends not only on the reflectance properties of viewed objects but also on the sensitivities of retinal cones, ambient light spectra, transmittance of ocular media, background reflectance, and receptor noise (Montgomerie 2006). Therefore, to assess whether differences caused by sunlight are visible to birds, we used color-discrimination models (Vorobyev and Osorio 1998, Vorobyev et al. 1998) that include all the above information. Moreover, color-discrimination models enabled us to test whether the magnitude of the change in color in various color regions differed.

METHODS

During May 2004, we collected the feathers of adult male and female bee-eaters of unknown age on Kinmen Island, in the Taiwan Strait ~5 km east of China's shore (118° 24′ E, 24° 27′ N) as a part of another study (field details described in Siefferman et al. 2007). Bee-eaters are socially monogamous, colonially nesting, aerial insectivores that forage near breeding sites (Burt 2002). During the breeding season, Blue-tailed Bee-eaters forage and socialize in open habitats with direct sunlight (Siefferman, pers. obs.).

After collecting feathers, we stored them in envelopes in the dark because long-term storage of feathers in the dark does not change their pigment composition (e.g., McGraw et al. 2003). Therefore, we are not concerned that the pigment content of feathers was altered prior to analysis. In April 2009, we taped feathers to matte black paper and measured them before the experiment. Using a USB4000 spectrometer and a pulsed xenon lamp (PX2) connected with a fibre-optic measuring probe (R 200-7-UV⁄VIS; Ocean Optics, Dunedin, FL, USA), we took five readings from each of three body regions (chin, back, and rump). Using a 90° angle, we fixed the distance from the feather surface at 1.5 mm and thus illuminated an area 2 mm in diameter. Before measuring each individual, we standardized measurements with a white standard (WS-1-SL, Labsphere, North Sutton, NH), while we set the dark standard by turning off the light and covering the probe.

We expressed spectral measurements as percentage of light per wavelength. We calculated color variables for each body region by the same procedure as in the previous study of Blue-tailed Bee-eater coloration (Siefferman et al. 2007). These include measurements of hue and chroma for the blue (rump), green (back), and yellow (chin) regions. We calculated chroma as the proportion of light reflected in the blue (400–510 nm) and green (510–605 nm) regions of the spectrum to the total reflectance (300–700 nm). We calculated the blue chroma to estimate the chroma of the yellow chin because absorption of carotenoids is greatest in this region (McGraw et al. 2001). We calcuated hue, the principal color reflected by the feather, as the wavelength of the peak of blue, green, and yellow in the relevant part of the plumage (H1 in RCLR software). We processed spectral data with RCLR v0.9.28 software (Montgomerie 2008).

After measuring the initial feather color we placed feathers on cards in two flat plastic boxes (600 × 600 × 5 mm) covered with a sheet of 2-mm UVD acrylic glass (Quinn

Plastics). This type of plastic has a high transparency to the wavelengths of UV light that reach the earth's surface, i.e., 290–400 nm (mean transmittance for 1 nm within this range is 87.3 ± 4.2%). We glued the lids tightly to the boxes with silicone to prevent samples from acquiring soil or moisture. We placed the boxes on the flat roof of the Faculty of Biology building at Adam Mickiewicz University in Poznań (52° 47′ N, 16° 92′ E) from 24 April to 1 June 2009. During periods of bad weather, boxes were kept inside the building. In total, samples were exposed to sunlight for 25 days (24 hr per day). This length of exposure corresponds roughly to the length of egg laying and incubation in the Blue-tailed Bee-eater. After the experimental exposure to sunlight, we measured the feathers again in the manner described above.

VISUAL MODELING

To assess how sunlight-induced changes in color are perceived by birds, we calculated chromatic (ΔS) and achromatic contrast (ΔL) of feather color before and after exposure. The chromatic contrast (ΔS) is expressed in a unit called the just-noticeable difference. Vorobyev et al. (1998) assumed that birds can distinguish ΔS values >1.0. A greater value of ΔS suggests a greater ability of a bird to detect the difference between two color patches. We calculated chromatic contrast (ΔS) in the following way. For average reflectance spectra from each region (i.e., yellow chin, blue rump, and green back) and for each individual, we computed cone quantum catches (Q_i) for each cone type by the formula of Vorobyev et al. (1998):

$$Q_i = \int_\lambda R_i(\lambda) S(\lambda) I(\lambda) O(\lambda)\, d\lambda$$

where λ = a wavelength, $R_i(\lambda)$ = the sensitivity of cone type i, $S(\lambda)$ = the reflectance spectrum, $I(\lambda)$ = the irradiance spectrum, and $O(\lambda)$ = the transmittance of the ocular media.

Members of the order Coraciiformes have four types of cones that are sensitive to very short (VS), short (S), medium (M), and long (L) wavelengths (Ödeen and Håstad 2003). Molecular analyses of opsin genes in VS cones in the Coraciiformes demonstrate that they are sensitive to violet light (peak sensitivity at 405 nm; Ödeen and Håstad 2003). Because the sensitivities of other cone types (i.e., S, M, L) have not been studied in any of the Coraciiformes, we used data from the chicken because it also possesses violet-sensitive VS cones (Govardovskii and Zueva 1977, Partridge 1989, Bowmaker et al. 1997). We used Endler's (1993) Blue Sky spectrum as the irradiance spectrum.

We calculated the discriminability of two spectra by the following equation:

$$\Delta S^2 = (\omega_1\omega_2)^2(\Delta f_4 - \Delta f_3)^2 + (\omega_1\omega_3)^2(\Delta f_4 - \Delta f_2)^2 + (\omega_1\omega_4)^2 (\Delta f_3 - \Delta f_2)^2 + (\omega_2\omega_3)^2(\Delta f_4 - \Delta f_1)^2 + (\omega_2\omega_4)^2(\Delta f_3 - \Delta f_1)^2 + (\omega_3\omega_4)^2(\Delta f_2 - \Delta f_1)^2 / [(\omega_1\omega_2\omega_3)^2 + (\omega_1\omega_2\omega_4)^2 + (\omega_1\omega_3\omega_4)^2 + (\omega_2\omega_3\omega_4)^2]$$

where $\Delta f_i = \Delta q_i / q_i$, q_i is cone quantum catch (Q_i) normalized for the irradiance spectrum, and ω_i represents receptor noise that depends on the scaling factor T, the relative abundance of cone types, and the Weber fraction for the cone type. Scaling factor relates a proportion of the maximal cone catch to an absolute cone catch. We set T to 10 000 that roughly corresponds to bright illumination. We used a Weber fraction of 0.05 for all cone types and the following relative abundance of cones from the Blue Tit (*Cyanistes caeruleus*): VS = 0.37, S = 0.70, M = 0.99, L = 1.00 (Hart et al. 2000).

The Vorobyev–Osorio model assumes that color discriminability does not depend on a color's brightness (Vorobyev et al.1998). We therefore calculated achromatic contrast (ΔL) by the formula of Siddiqi et al. (2004), $\Delta L = \Delta f_i / \omega$, where

$$\Delta f_i = \ln[q_i(\text{spec1})/q_i(\text{spec2})]$$

and q_i indicates double cone quantum catches for two reflectance spectra (spec1 and spec2). Double cones are assumed to be involved in achromatic vision (reviewed in Cuthill 2006). We used double cone sensitivities data provided by Hart et al. (2000). Siddiqi et al. (2004) considered two reflectance spectra differing by more than 1.0 (ΔL values > 1.0) to be distinguishable by birds.

We calculated cone quantum catches and chromatic discriminability with SPEC.01 software (Hadfield 2004).

STATISTICAL ANALYSIS

We used Statistica 8.0 software to analyze data, and all statistical tests were two-tailed. We tested for normality with Shapiro–Wilk tests, the effect of sunlight on color change with paired Student's *t*-tests (differences of individual samples before and after the treatment). Values of chromatic and achromatic contrast were not normally distributed, even after transformation (Shapiro–Wilk test, $P < 0.05$ for all cases). Therefore we used a Friedman ANOVA to test differences in contrast among three plumage regions within one individual. To assess the repeatability (Lessells and Boag 1987) of spectrometer measurements, we measured a subset of sun-exposed feathers from 28 individuals again 5 months later. During that time between measurements, feathers were kept in tightly sealed plastic bags, in the dark in a wooden chest.

The repeatabilities (R) of all color parameters were significant: blue chroma: $R = 0.40$, $F_{1,27} = 3.49$, $P < 0.01$; blue hue: $R = 0.50$, $F_{1,27} = 4.80$, $P < 0.01$; green chroma: $R = 0.29$, $F_{1,24} = 1.96$, $P = 0.04$; green hue: $R = 0.44$, $F_{1,24} = 4.00$, $P < 0.001$; yellow chroma: $R = 0.43$, $F_{1,27} = 3.86$, $P < 0.001$; yellow hue: $R = 0.57$, $F_{1,27} = 6.00$, $P < 0.001$.

RESULTS

Only the hue of the blue rump was unaffected by exposure to sunlight (Table 1, Fig. 1). The blue chroma metric of these feathers decreased with sunlight, indicating they became less colorful (Table 1, Fig. 1). The green back feathers decreased in

TABLE 1. Comparison of variables quantifying the color of Blue-tailed Bee-eater feathers before and after exposition to sunlight. Values are mean ± SD.

Body region and color trait	Before exposure	After exposure	t	df	P
Green back					
Chroma	0.40±0.03	0.38± 0.03	5.4	50	<0.01
Hue	575.3 ±6.4	579.7 ± 7.3	–4.4	50	<0.01
Blue rump					
Chroma	0.32±0.020	0.31± 0.02	3.1	54	<0.01
Hue	532.1 ±9.7	532.8 ±12.1	–0.5	54	0.62
Yellow chin					
Chroma	0.17±0.03	0.18± 0.03	–4.5	50	<0.01
Hue	579.3 ±7.0	577.3 ± 7.6	3.3	50	<0.01

the green chroma metric and shifted to a hue of longer wavelength, also suggesting a less colorful state (Table 1, Fig. 1). The yellow carotenoid-based plumage of the chin increased in the blue chroma metric and shifted to a lower hue, again implying a less colorful state (Table 1, Fig. 1).

The median values (median, 25%–75% percentiles) of the chromatic contrast (ΔS) in all three regions were <1.0 [yellow: 0.70 (0.42–1.25), green: 0.85 (0.49–1.09), blue: 0.54 (0.32–0.88)]. Regions did not differ significantly when values of contrast were compared within the same individual (Friedman ANOVA, n = 51, $\chi^2 = 4.39$, df = 2, $P = 0.11$). The number of individuals in which chromatic contrast exceeded 1.0 in each plumage region was as follows: yellow chin, 37% ($n = 19$); blue rump, 16% ($n = 8$); green back, 33% ($n = 17$). The proportion of individuals with $\Delta S > 1.0$ to those with $\Delta S < 0.1$ differed significantly by plumage region (chi-squared test, $\chi^2 = 6.57$, df = 2, $P = 0.04$).

Values of achromatic contrast (ΔL; median, 25%–75% percentiles) calculated for the three color regions were as follows: yellow chin, 4.10 (1.51–7.30); green back, 1.62 (0.62–3.57); blue rump, 1.41 (0.82–2.71). Differences among plumage regions were statistically significant when values for contrast were compared within the same individual (Friedman ANOVA, n = 51, $\chi^2 = 10.63$, df = 2, $P = 0.005$; Fig. 2). The achromatic contrast of yellow chin feathers was >1.0 in 78% (40/51) of the individuals, while 62% (35/51) of the individuals had green back and blue rump feathers in which the achromatic contrast was >1.0. The proportion of individuals with $\Delta L > 1.0$ to individuals with $\Delta L <$ 0.1 did not differ significantly by plumage region (chi-squared test, $\chi^2 = 1.62$, df = 2, $P = 0.45$).

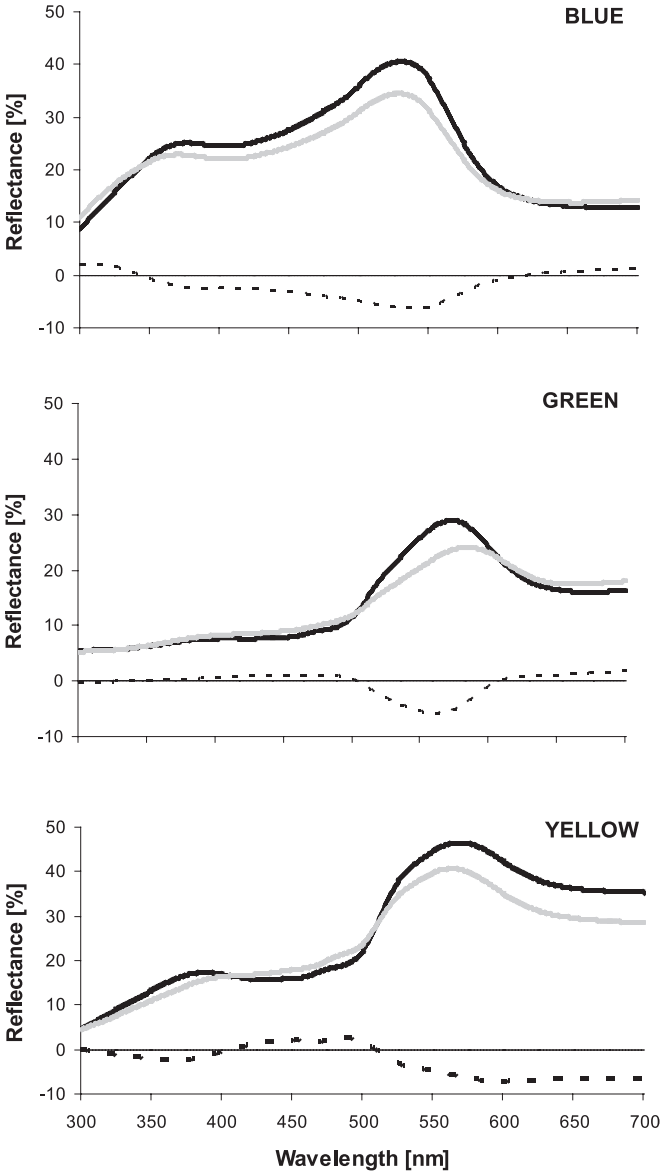

FIGURE 1. Reflectance spectra of typical blue, green, and yellow regions of the Blue-tailed Bee-eater. Black lines represent intact feathers, gray lines feathers after exposure to the sunlight. Dotted lines below indicate changes in reflectance due to the experiment.

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DISCUSSION

All three regions of Blue-tailed Bee-eater plumage we studied (yellow, green, and blue) responded to experimental exposure to solar radiation. In general, the effects of sunlight appeared to cause a reduction in the reflectance of long wavelengths of light, suggesting changes to the underlying feather microstructure greater than those to carotenoid pigments. Moreover, although reflectance-spectrometry data suggest that these changes should make birds less colorful, consideration of models of avian vision indicates that the changes in plumage brightness, but not those in chroma and hue, are likely visible to the bee-eaters. The models suggest that the changes to the yellow (carotenoid-based) coloration should be the most obvious to the birds.

One limitation of our experimental design was that we did not incorporate a true control group; we did not measure

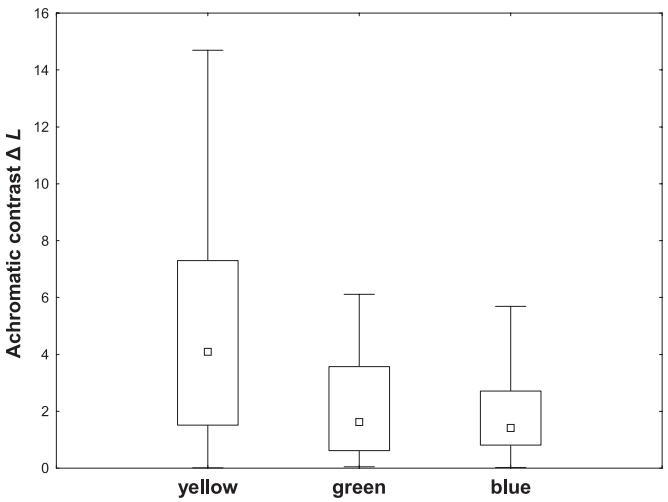

FIGURE 2. Values of chromatic contrast (ΔL) between intact feathers and the same feathers after 25 days of exposure to sunlight. Shown are medians (points), 25–75% percentiles (bars), and minima and maxima (whiskers).

unexposed feathers before and after 25 days. Nonetheless, we are confident that factors other than sunlight did not contribute to our results. First, feathers were kept in tightly closed boxes throughout the experiment, so we excluded effects of soiling, abrasion, or rapid changes of moisture. Second, the frequent exposure to direct full-spectrum sunlight should have caused extremely unfavorable conditions for growth of keratophilic bacteria and other microbes that could have affected the feathers' colors. Third, although high temperature may increase the rate of photo-oxidation of carotenoids, it is not likely an important factor responsible for that process (Christophersen et al. 1991). A previous sunlight-bleaching experiment demonstrated that carotenoid-pigmented feathers stored in the dark maintain their coloration despite being exposed to high temperature similar to those of our experiment (Surmacki 2008). It is also unlikely that temperature affects structural coloration via keratin degradation; feather keratin has very high thermal stability (Takahashi et al. 2004). Degradation of feather keratin starts at 110 °C (Takahashi et al. 2004), while the maximal temperatures recorded during our experiment were ~50 °C (Surmacki, unpubl. data).

We predicted that changes in the yellow carotenoid-based feathers would mimic variation due to varying concentrations of carotenoids in feathers. Both theoretical models and empirical studies show that carotenoids absorb mainly blue wavelengths of light. Therefore, a decrease in pigment concentration should result in an increase in reflectance between 400 and 500 nm (MacDougall and Montgomerie 2003, Andersson and Prager 2006). Moreover, a lower carotenoid concentration should cause a hue to shifts toward shorter wavelengths (Andersson and Prager 2006, Saks et al. 2003). In our study, we observed an increase in the chroma of the yellow feathers (i.e., relative reflectance between 400 and 510 nm). However, this change was likely caused by a combination of a slight increase in reflectance in the blue wavelengths coupled with a marked decrease in the reflectance of longer wavelengths (500–700 nm; Fig. 1). It is likely that reflectance in the UV and short wavelengths in the Blue-tailed Bee-eater's feathers are governed more by the organization of the keratin nanostructure than by carotenoid content (see Shawkey and Hill 2005), which may explain the relatively small changes in these regions. Another possibility is that sunlight-induced oxidation affected mainly the keratin's microstructure and this, in turn, caused changes in coloration.

The reflectance of light from the Blue-tailed Bee-eater's green plumage likely depends on a combination of tissue nanostructure and carotenoid pigments (Siefferman et al. 2007), while the turquoise-blue color is caused by feather nanostructure alone. We have used pyridine extractions (see methods in McGraw et al. 2005) to attempt to isolate carotenoid pigments from bee-eater feathers. Preliminary results suggest the presence of the same carotenoid pigments (xanthophylls) in yellow and green feathers but no evidence of carotenoids in the turquoise-blue feathers (Pannkuk and Siefferman, unpubl. data). Our data corroborate failed attempts to extract carotenoid pigments from the similarly colored turquoise-blue feathers of another coraciiform, the European Roller (*Coracias garrulus*; Silva et al. 2008).

After exposure to sunlight, reflectance of the green and yellow feathers decreased at similar wavelengths (particularly, 500–600 nm; Fig. 1). In the Green Jay (*Cyanocorax yncas longirostris*), in which the green of the plumage is produced by both carotenoids and nanostructure, green feathers change to blue over time, presumably because of the destruction of pigments by sunlight (Johnson and Jones 1993). In our study, however, green hue shifted toward longer wavelengths, the opposite of what might be expected after a reduction of carotenoid concentration (see results for yellow feathers). Therefore, as for yellow feather coloration, the disturbance of keratin microstructure caused by sunlight seems to be responsible for changes in green feather coloration.

For the turquoise-blue rump coloration, we found a change in chroma but not in hue, and this appears to be driven by an overall reduction in reflectance of wavelengths of light, particularly from 500 to 600 nm (Fig. 2). Structural coloration is not expected to be affected by sunlight (Hill 2006) except if the feather cortex is ruptured, causing the loss of barbules, as has been observed from inoculation of feather-eating bacteria (Shawkey et al. 2007). Another hypothesis, however, argues that irradiation with sunlight could modify the nanostructure of keratin and thus change pigment-based feather coloration (Brush 1990). Sunlight (UV radiation) is hypothesized to denature keratin and other proteins and thus protect pigments from degradation. Blanco et al. (2005) proposed this mechanism as an explanation for a shift in the carotenoid-based nuptial plumage of the Linnet (*Carduelis cannabina*) from duller to brighter. If sunlight modifies keratin organization in barbs,

it may also change the coherent reflection of light from structurally based plumage coloration. Unfortunately, this hypothesis has never been tested by analysis of the microstructural anatomy of feather barbs.

Our analyses of chromatic contrast (ΔS) suggest that changes in the shape of the reflectance curves (hue and chroma) were generally not visible to the Blue-tailed Bee-eater. The percentage of individuals in which the contrast exceeded the perceivable threshold of 1.0 varied from 16 to 37%, depending on the plumage region. On the other hand, in all three body regions, achromatic contrast, which describes changes in overall brightness, was visible ($\Delta L > 1.0$) in the majority of individuals. Visual modeling revealed some differences between color types in their response to sunlight. More than twice as many individuals reached a perceivable level of color change ($\Delta S > 1$) for the yellow and green regions than reached it for the blue region. Similarly, achromatic contrast (ΔL) was the highest for yellow feathers. These findings suggest a gradient in sensitivity to sunlight-induced change in coloration that is visible to birds, with structural colors (blue) being the most resistant to sunlight, carotenoid-based (yellow) the most sensitive, and mixed (green) intermediate in sensitivity.

In the only other study that focused on the effect of sunlight exposure on feather color (Surmacki 2008), the effect was markedly more pronounced. Values of chroma and hue of the yellow feathers of the Great Tit shifted 52% and 5%, respectively, when exposed for 31 days, and these changes were easily visible to the human eye (Surmacki 2008; Surmacki, unpubl. data). In comparison, the chroma and hue of the yellow chin feathers of the Blue-tailed Bee-eater changed by only 6% and 0.3%, respectively. The reason for this discrepancy might be differences in feather structure that reflect adaptation to local conditions. Blue-tailed Bee-eaters live in open habitats and a tropical climate, where potential exposure to sunlight is much higher than in the temperate woodland habitat of the Great Tit. It is possible that Blue-tailed Bee-eater feathers have evolved adaptations to counteract the destructive effects of intense sun radiation.

Multiple important conclusions emerged from our study. First, sunlight influenced each type of coloration of Blue-tailed Bee-eater plumage (structural, carotenoid-based, and mixed). Second, all of these changes in plumage caused the bee-eaters to be less colorful. Third, changes in yellow and green colors seem to be influenced less by carotenoid breakdown and more by modifications of keratin microstructure caused by photo-oxidation. Finally, the visual models revealed that, in most individuals, these plumage changes were below the birds' level of perception. Analysis of chromatic and achromatic contrasts also showed that changes in purely structural colors (blue feathers) were less obvious to the birds and thus are more resistant to the effects of sunlight than are those based on carotenoid pigments (yellow and green feathers). Our results, however, should be treated with caution because we did not test the mechanism by which each color region was affected by solar radiation. To reveal the mechanisms of sunlight-induced color changes, analyses of both pigment content and keratin nanostructure are needed. Furthermore, experiments with different periods of exposure could provide information about the minimum time needed for sunlight to cause visible changes in plumage coloration. Similarly, the location of the experiment could also affect the results. In the tropics, the effect of sunlight should be greater than in the temperate zones. Our results, therefore, should be conservative because, in the tropics, rays of sunlight reach the ground at an angle more perpendicular than in the temperate zone. These changes in plumage coloration caused by sunlight could have consequences for bee-eaters' fitness. An earlier study of this population demonstrated that the species is sexually dichromatic and chromatic variation in plumage coloration is correlated with body condition (Siefferman et al. 2007), suggesting that plumage colors may play a role in sexual signaling. Although the feathers used in this experiment were taken from birds of unknown sex, our data demonstrate that sunlight degrades carotenoid-based and structural color. Most of the effects on chroma, however, should not have been visible to the birds. Thus shifts in plumage color during the breeding season may not have significant effects on the breeding success of their bearer, e.g., by influencing decisions concerning social and extra-pair mating (Safran et al. 2005). Future research with this population is necessary to understand the signaling function of variation in plumage coloration.

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