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METHODS

A novel method for quantifying the glossiness of animals

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Abstract The glossy sheen of healthy hair is an ideal of human beauty; however, glossiness has never been quantified in the context of non-human animal signaling. Glossiness, the specular reflectance characteristic of polished surfaces, has the potential to act as a signal of quality because it depends upon material integrity and cleanliness. Here, we undertook two studies of glossiness in avian plumage to determine (a) the repeatability of a recently developed measure of glossiness, (b) the relationship between glossiness and conventional measures of coloration, and (c) how glossiness is associated with quality signaling. Using museum specimens of three North American bird species with glossy plumage (red-winged blackbird, *Agelaius phoeniceus*; great-tailed grackle, *Quiscalus mexicanus*; Chihuahuan raven, *Corvus cryptoleucus*), we found that the glossiness measure was highly repeatable for all species and was significantly correlated with plumage coloration (e.g., chroma, brightness) in male great-tailed grackles. We then used wild-caught grackles to examine sexual dimorphism in plumage glossiness and its correlation to a potentially sexually selected trait in this species, male tail length. We found that males were significantly glossier than females and that male, but not female, glossiness correlated positively with tail length. This study provides a repeatable method to measure glossiness and highlights its potential as a signal of individual quality in animals.

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Introduction

Visual signals in animals are the subject of intense study by behavioral ecologists and have provided important insights into many evolutionary processes, including sexual selection, crypsis, aposematism, and mimicry (Andersson 1994; Ruxton et al. 2004). Animal coloration, in particular, has received a great deal of attention, and its study has accelerated of late with the development of new technologies and techniques. Early studies of animal coloration often involved subjective rankings of color variation by human observers, but with the advent of portable and affordable reflectance spectrophotometers, researchers began using much more objective color metrics (reviewed in Andersson and Prager 2006). Spectrophotometry has allowed researchers to observe variation hidden to the human eye (Bennett et al. 1994) and, with appropriate physiological data, create species-specific measures of visual stimulation (e.g., Vorobyev et al. 1998). Although these approaches are an excellent way to objectively measure coloration, it has proven difficult to measure and compare the coloration of animals in a holistic way that accounts for interactions among aspects of the visual signal (e.g., Endler and Mielke 2005). For human observers, such interactions are the basis of the perception of surface properties like roughness, gloss, and translucency (Landy 2007). To date, essentially no rigorous quantitative approaches have been taken to understand these complex axes of perceptual variation in the visual signals of non-human animals.

In this study, we have applied an image statistical approach recently developed by Motoyoshi et al. (2007) to quantify the glossiness of bird plumage and examine its relationship to other signaling traits. Here, we define glossiness as the quality of mirror-like or specular reflectance characteristic of a smooth polished surface (Andersson and Prager 2006; Landy 2007). The integument of many animals is characterized as glossy; examples include the shiny elytra of beetles (Hegedüs et al. 2006), the oily fur of mustelids (Rasmussen and Dyck 2000), and the sleek black plumage of blackbirds (Jaramillo and Burke 1999). Glossiness (also referred to as luster or sheen) of human hair has been suggested as an indicator of health and age (Gangestad and Scheyd 2005) and has great potential as a signal in animals because it depends upon the structure, order, integrity, and cleanliness of the integument (Stamm et al. 1977; Rasmussen and Dyck 2000; McMullen and Jachowicz 2003), which may be costly to produce and/or maintain. However, investigating glossiness is challenging because it cannot be captured from typical reflectance measurements. Objects having identical mean luminance values can differ considerably in their perceived glossiness (Motoyoshi et al. 2007). Recently, Motoyoshi et al. (2007) and Sharan et al. (2008) demonstrated that the perceived glossiness of a surface is related to the statistical distribution of luminance values within a scene (i.e., skewness), providing a new tool to investigate this phenomenon.

The goals of our study were to: (1) evaluate the utility and repeatability of this new skewness measure for quantifying animal glossiness, using bird plumage as a model; (2) compare glossiness to conventional measures of color (e.g., hue, chroma, brightness) in avian feathers, to determine whether glossiness is capturing something fundamentally different about a visual signal than traditional tristimulus color metrics; and (3) investigate the signaling potential of glossiness by examining its correlations with potentially sexually selected traits and individual condition in the great-tailed grackle, *Quiscalus mexicanus*. To tackle the first two objectives, we measured plumage reflectance properties from museum specimens of three North American bird species that are all considered glossy (red-winged blackbird, *Agelaius phoeniceus*; great-tailed grackle; Chihuahuan raven, *Corvus cryptoleucus*; Fig. 1a–c). To examine the relationship between glossiness and potentially sexually selected traits and condition, we captured wild great-tailed grackles (see more below) and examined how glossiness differed between the sexes and correlated with measures of body size and tail length as well as measures of health and condition (blood parasitemia and heterophil to lymphocyte (H/L) ratio). Blood parasite prevalence has been shown to be associated with plumage color in the common grackle (Kirkpatrick et al. 1991), and H/L ratio is considered indicative of stress (Vleck et al. 2000; Bonier et al. 2007).

Materials and methods

Museum specimens

In May 2009, we collected plumage photographs and reflectance spectra measurements (details below) from grackle, blackbird, and raven specimens at the Museum of Southwestern Biology at the University of New Mexico in Albuquerque, NM, USA (Table 1). These species were chosen because they vary in their level of iridescent coloration. Iridescence and glossiness are distinct phenomena but not mutually exclusive. Many species are described as glossy iridescent (e.g., Doucet et al. 2006), and we know a great deal about the mechanisms and functions of iridescent coloration (Meadows et al. 2009). With our three study species, we wanted to examine how our measurement of glossiness is related to established measures of plumage iridescence and if glossiness is a property distinct from iridescence. Red-winged blackbirds are characterized as having glossy black plumage (Jaramillo and Burke 1999; Yasukawa and Searcy 1995), but are considered non-iridescent (matte; Shawkey et al. 2006). Male great-tailed grackles have glossy black plumage with distinct bluish-green iridescence (Jaramillo and Burke 1999; Johnson and Peer 2001; Shawkey et al. 2006). Both male and female Chihuahuan ravens have glossy black plumage, and Bednarz and Raitt (2002) indicate that they may have subtle iridescence; however, based upon our qualitative impressions (i.e., apparent change in hue with viewing angle) and the criteria of Shawkey et al. (2006), the birds in our sample were not iridescent. Fading and wear are known to degrade plumage coloration (McNett and Marchetti 2005) and could have similar effects on glossiness. To test for the effects of specimen age, we recorded the age of each specimen and calculated the Pearson's correlation coefficients between glossiness and age for each species.

Wild bird capture and sampling

The great-tailed grackle is a blackbird species (Family Icteridae) with a polygynous mating system and marked sexual dimorphism in body size, tail length, and plumage appearance. Male grackles have glossy iridescent black plumage and a long tail, whereas females are drab brown with shorter tails. Comparative studies suggest that male body size and tail length are sexually selected traits (Björklund 1991; Webster 1992), and these traits are

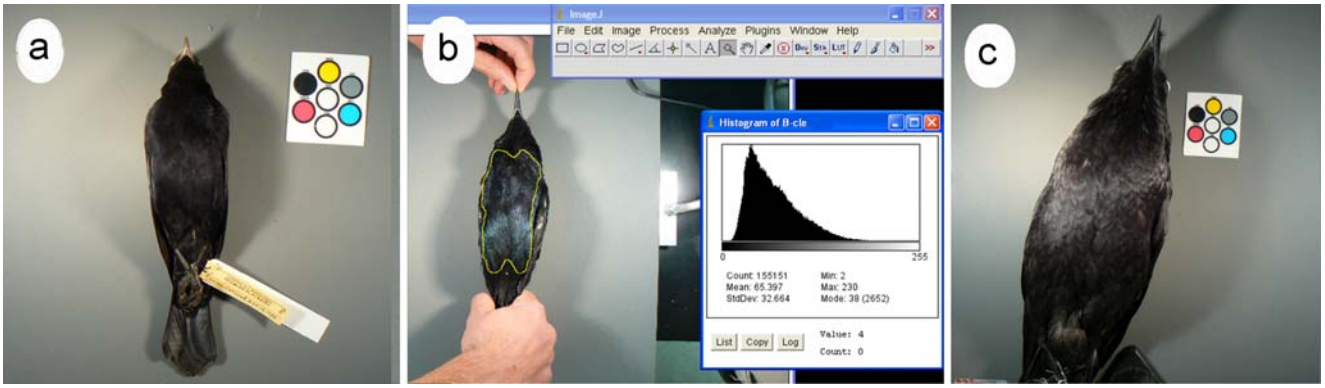

Fig. 1 **a** Red-winged blackbird, **b** great-tailed grackle, and **c** Chihuahuan raven study skins photographed for this study. **b** A screen capture of the image analysis process. The area circled in *yellow* is the pixel selection for analysis, and the *histogram* represents the distribution of luminance values within the selection. The distribution is asymmetrical and positively skewed toward higher luminance values, which is diagnostic of glossy surfaces (Motoyoshi et al. 2007)

associated with territory holding and the frequency of extra-pair fertilizations in breeding great-tailed grackles (Johnson et al. 2000). Males display their plumage in stereotyped fashion during agonistic and courtship encounters, where they alternately sleek and ruffle their shiny feathers (Johnson and Peer 2001). Subjective estimates of plumage reflectance (Johnson and Peer 2001) and reproductive success (Johnson et al. 2000) increase with age in male *Q. mexicanus*. Therefore, glossiness may be a visual signal in this species, and we predicted that it would correlate positively with aspects of sexually selected body size and tail length. This species was chosen because it is glossy, sexually dimorphic, and abundant in our local area. However, we recognize that great-tailed grackles vary not only in glossiness, but also in traditional measures of color that correlated with glossiness (see below) and may confound our analysis. We therefore present this study as an example of the applicability of our glossiness methods and a starting point for further investigations, rather than a definitive test of signaling function. From 20 January–22 May 2008, we captured nine adult female, five after-second-year (ASY) male and eight second-year (SY) male great-tailed grackles in Tempe, Arizona, USA with a modified Australian crow trap (Johnson et al. 2000). Males were aged by plumage characteristics (Johnson and Peer 2001), and we measured body mass, wing chord, tail length, tarsus length, and bill size. We also collected three breast feathers from each bird for reflectance spectrophotometry and photographed them under standardized conditions (see below) to measure glossiness. We collected a 100 µl blood sample from the alar vein using heparinized capillary tubes to measure two hematological parameters that are often associated with health state: the prevalence of blood parasites and white blood cell quantification. We determined blood parasitism (the proportion of total individuals infected with *Plasmodium*, *Haemoproteus*, *Trypanosoma*, or *Microfilariae*), and the ratio of heterophils to lymphocyte (H/L) following the methodology of Fokidis et al. (2008). H/L ratio increases in response to stressors such as malnutrition and injury

Table 1 Museum specimen sample size, age, glossiness (skewness), variation in glossiness, and within individual repeatability of the glossiness measurement

Species	Sample size	Mean specimen age, years (range)	Mean skewness ±SE	Coefficient of variation skewness (range)	Repeatability			
					F	*df*	*p*	*R*
Red-winged blackbird	15 male	58 (13–106)	0.95±0.074[a]	33.52 (−0.11–2.22)	18.01	13,28	<0.0001	0.85
Great-tailed grackle	27 male	38 (15–43)	1.12±0.055	26.65 (0.56–1.79)	24.56	27,53	<0.0001	0.89
Chihuahuan raven	6 male, 4 female	33 (9–39)	1.24±0.090[a]	20.79 (0.90–1.71)	135.02	15,32	<0.0001	0.98

[a] Differ significantly (Fisher's LSD post hoc comparison, $p<0.05$)

and has been suggested as an indicator of chronic stress (Vleck et al. 2000; Bonier et al. 2007).

Glossiness measurement

To assess plumage glossiness in both museum specimens and wild birds, we digitally photographed the breast of each bird in triplicate against a standardized gray background and under standard lighting conditions. The camera (Panasonic DMC-FZ7, Secaucus, NJ) was mounted 70 cm at a 90° angle above the bird, and two light fixtures (Phillips Natural Light 50 W, 120 V; Andover, MA) were located 30 cm away at a 45° angle. The white balance was set at the beginning of the study under the standard lighting conditions while focusing on the gray board background (18% gray, Testrite, Newark, NJ). We used an F-number of F/3.2, exposure time of 1/30 s, and ISO speed of 100, and images were saved in the uncompressed Tagged Image File Format (TIFF). Birds were positioned on their backs with both tarsi held flush against the tail and with the beak held parallel to the back and the crown facing downward (Fig. 1a–c). We repositioned the birds between each photo to assure independence among photographs.

The output of most conventional digital cameras, like ours, is non-linear with respect to light intensity, which can compromise the usefulness of measurements taken from digital photos (Stevens et al. 2007). To correct this non-linearity, we linearized the pixel intensity values (mean of red, green, and blue channel output) with respect to a gray scale standard (Kodak gray scale, Tiffen Co., Hauppauge, NY). We directly measured the mean reflectance (300–700 nm) of each of the 20 gray density steps on the gray scale standard with a reflectance spectrophotometer (see below), then photographed the gray scale four times under the conditions described above. Using ImageJ software (Abramoff et al. 2004), we measured the mean pixel intensity from each of the gray density steps in the images and then fit these pixel intensities to the direct measures of reflectance. The following transform of pixel intensity (p) from our camera provided a good fit (r^2=0.9984) to the directly measured reflectance values:

$$\text{reflectance} = 0.00729\left(e^{\left(\frac{p}{34.002}\right)}\right)$$

We analyzed the bird images using ImageJ by selecting the breast contour feathers with the freehand selection tool and measuring the pixel intensity of the selection with the histogram function (Fig. 1b). We converted the pixel intensities to reflectance values with the equation given above, log-transformed these values (as suggested by Sharan et al. 2008) and calculated skewness (hereafter referred to as glossiness) of the log-reflectance distribution as follows (Motoyoshi et al. 2007; Sharan et al. 2008):

$$\text{SD} = \sqrt{\frac{\sum^{(I(x,y)-m)^2}}{N}}$$

$$\text{skewness} = \frac{\sum (I(x,y)-m)^3}{N(\text{SD})^3}$$

where $I(x,y)$ is the log-reflectance of the pixel located at coordinates x,y within the selection, m is the mean log-reflectance of the selection, and N is the total number of pixels selected. From the three photographs of each individual, we calculated repeatability of our skewness measurement following Lessells and Boag (1987) and mean skewness, which we used in subsequent statistical analyses.

Reflectance spectrophotometry

We measured plumage reflectance (300–700 nm) of museum specimens using an Ocean Optics S200 spectrophotometer (Ocean Optics, Dunedin, FL) illuminated with a pulsed-xenon light source (PX-2, Ocean Optics Inc., Dunedin, FL) relative to a white standard (Spectralon 99% white standard, Labsphere, North Sutton, NH, USA). We took measurements at three points on the breast plumage (one along the midsagittal plane and one approximately 3 cm to either side of the plane) with the probe at a 45° angle and a distance of 1 cm from the plumage surface.

Reflectance data from wild-caught great-tailed grackles were gathered using a different methodology that allowed us to account for the angle dependence of iridescence and to measure reflectance with a probe at an equal angle to the light source, thus avoiding potential error caused by small differences in feather orientation or compression. We were unable to use this more rigorous method on museum specimens because we could not pluck feathers from them. Three breast feathers from each wild grackle were plucked from the midsagittal plane as described above and individually taped by the rachis to matte-black card stock. A mounted feather was placed on a translational stage (Thorlabs, Newton, NJ) in a dark room and illuminated with a pulsed-xenon light source (PX-2, Ocean Optics Inc., Dunedin, FL) via a fiber-optic cable (400 μm diameter, Ocean Optics) focused to a 10 mm diameter area with a collimating lens (74-UV, Ocean Optics) at 70° elevation with the rachis of the feather pointed towards the light source. A spectrophotometer (USB2000, Ocean Optics, Dunedin, FL) collected spectral data via a separate fiber-optic cable focused with a collimating lens to a measuring spot of 2 mm at an elevation of 70° on the opposite side.

The translational stage compensates for sample thickness and allows adjustment of the angle of the specimen to accommodate small differences in the angle at which feathers are maximally reflective; however, all of the grackle feathers in this study reflected maximally with the feather nearly horizontal. Spectra were measured relative to a magnesium oxide white standard (Kemp and Macedonia 2006). We averaged the three spectra collected from each specimen and calculated total brightness (B1, sum of reflectance 300–700 nm), two measures of hue (H1, wavelength of peak reflectance and H5, wavelength at maximum slope for spectra without a distinct reflectance peak), spectral purity (S4, maximum slope of the spectra curve), and UV/blue chroma (the sum of reflectance 300–500 nm divided by the sum of the total reflectance 300–700 nm). These color measures were calculated using CLR 1.05 and RCLR (Montgomerie 2008); the abbreviations given above correspond to those given in Montgomerie (2006).

Statistical analyses

For museum specimens, we compared plumage glossiness among the three species using a univariate analysis of variance (ANOVA). We calculated Pearson's correlation coefficients to examine relationships between glossiness and each of our spectral measures of feather coloration. Many of our color variables were significantly intercorrelated, but rather than collapse these into a single variable, we chose to analyze them separately to facilitate comparisons beyond this study. For these five correlations of color and glossiness, within each species/sex, we used a Bonferroni corrected significance level of $\alpha=0.01$.

For wild-caught grackles, we reduced measures of body size (mass, tarsus, wing chord, and bill dimensions) into two principal components (Body Size PC1 and PC2) that explained 42% and 27% of the variation in males and 49% and 22% in females, loading primarily for structural size and body mass, respectively. Tail length was not significantly correlated with either body size PC in males (Pearson's $-0.11 \leq r \leq 0.44$, $p \geq 0.14$), but significantly correlated with PC1 in females (Pearson's $r=0.73$, $p=0.027$).

We used ANOVA to compare glossiness differences among grackle females, SY males, and ASY males. Because of the marked sexual dimorphism in body size, tail length, and plumage appearance, we analyzed data for males and females separately in subsequent analyses. To examine the relationship between glossiness and tail length, we used analysis of covariance (ANCOVA) with body size PC 1 and 2, tail length, and age class as independent variables and date of capture as a covariate. Non-significant interaction terms were removed from the ANCOVA model. Because color and glossiness covary, we repeated the above ANCOVA, substituting color metrics for glossiness to provide a comparison between these aspects of visual appearance. We used Pearson's correlation analyses to determine relationships between glossiness, color measurements, and hematological parameters. All of our data met the assumptions of parametric statistics, and we used an alpha level of 0.05 unless otherwise indicated.

Results

Repeatability and species comparisons

Our measurement of glossiness was highly repeatable within individuals for museum specimens (Table 1) and wild-caught great-tailed grackles of both sexes ($F_{15,32}=168.78$, $p<0.0001$, $R=0.98$), suggesting that our measurement technique was reliable and repeated handling of the specimens did not affect glossiness. Mean glossiness differed, though not significantly, among species ($F_{2,49}=3.09$, $p=0.054$, Table 1, Fig. 3a) with ravens glossier than blackbirds in an uncorrected post hoc comparison. The glossiness of male ravens (mean±SE skewness, 1.29±0.12) did not differ significantly from female ravens (1.16±0.11;

Table 2 Pearson's correlation coefficients for plumage glossiness and spectral color measurements. Statistically significant correlations ($p \leq 0.002$) are denoted in italics

Species	Color measurement				
	Brightness (B1)	Hue (H1)	Hue (H5)	Saturation (S4)	UV/blue chroma
Red-winged blackbird ($n=15$)	−0.030	0.059	0.21	0.11	0.19
Chihuahuan raven ($n=10$)	0.15	0.033	−0.25	0.037	0.32
Museum male great-tailed grackle ($n=27$)	*0.47*	−0.36	−0.043	*0.43*	*0.44*
Wild-caught male great-tailed grackle ($n=13$)	*0.77*	−0.54	−0.52	*0.68*	*0.72*
Wild-caught female great-tailed grackle ($n=9$)	−0.29	0.43	0.12	−0.35	0.083

t=0.74, df=8, p=0.48). Within each species, glossiness varied considerably among individuals (Table 1), but was not significantly correlated with specimen age in any species (all Pearson's $-0.341<r<0.09$, $p>0.27$).

Glossiness and traditional measures of color

Plumage glossiness was positively correlated with measures of brightness and chroma in wild and museum-specimen male great-tailed grackles (Table 2), indicating that glossier males had brighter plumage with greater short-wavelength reflectance. There were no significant correlations between spectral color measurements and glossiness in blackbirds or ravens (Table 2). Plumage iridescence was not required to produce a glossy appearance, as non-iridescent ravens and the majority of blackbird specimens (ten of 15) lacked chromatic coloration (Fig. 2), but had the same levels of gloss as the great-tailed grackles (Table 1). Wild-caught male grackles were significantly glossier than museum specimens (t=4.6, df=37, $p<0.0001$, Fig. 3a).

Predictors of glossiness in wild-caught great-tailed grackles

Glossiness differed significantly between male and female great-tailed grackles ($F_{2,19}$=71.55, $p<0.0001$, Fig. 3a), with males being glossier. ASY and SY males did not differ significantly in glossiness (Tukey's post hoc, $p>0.05$). The sexually selected tail length of male wild grackles was a significant predictor of glossiness (Table 3), and males with longer tail feathers had glossier breast plumage (Fig. 3b). Glossiness of male grackle museum specimens was also significantly positively correlated with tail length (Pearson's r=0.51, p=0.0072, Fig. 3b). Age, sampling date, and body size were not significant predictors of male plumage glossiness (Table 3). Plumage coloration (brightness and UV/blue chroma) was significantly positively correlated with glossiness, but these conventional measures of plumage coloration were more strongly positively related

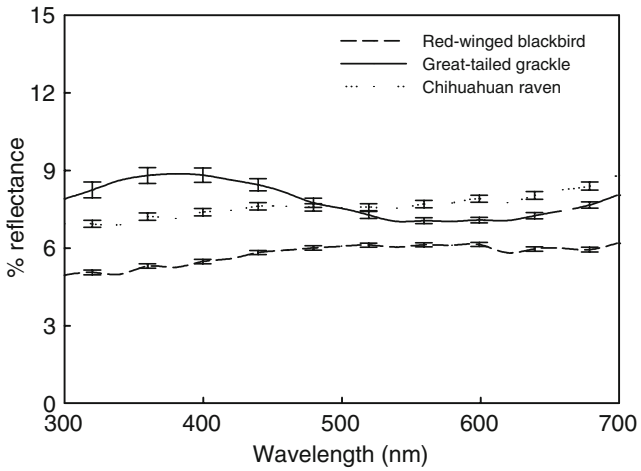

Fig. 2 Mean±SE plumage reflectance spectra of the three study species. Note that only the great-tailed grackle has a distinct reflectance peak

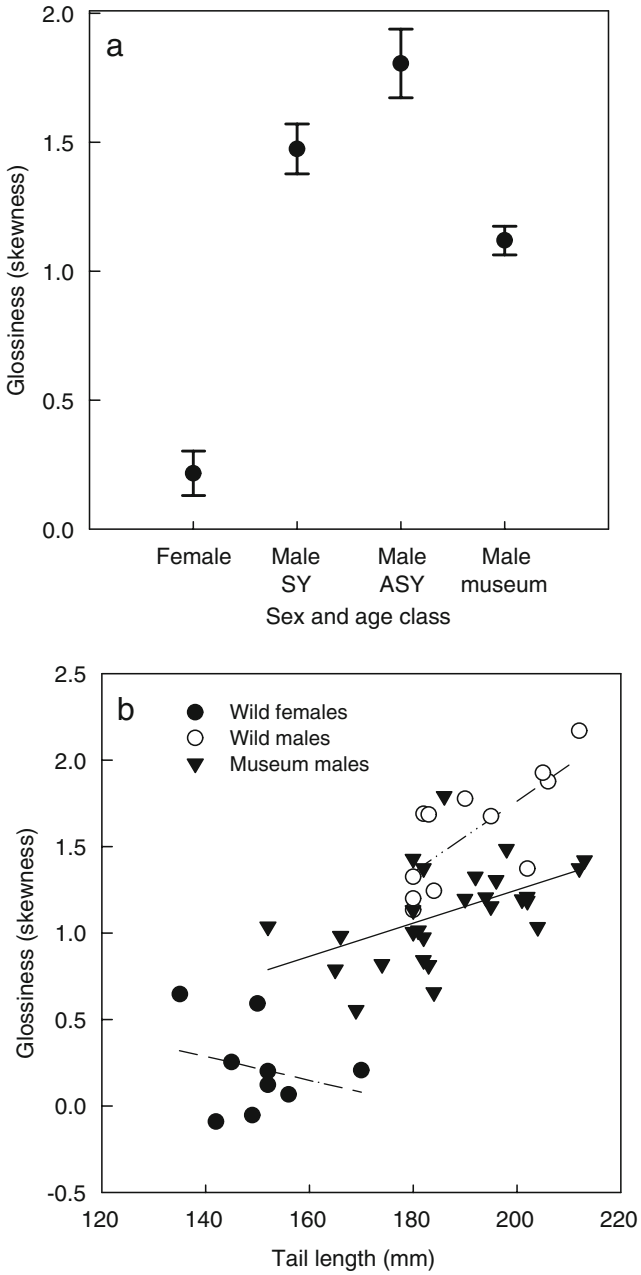

Fig. 3 **a** Mean±SE glossiness (skewness) of different age classes and sexes of wild-caught and museum-sampled great-tailed grackles. Female great-tailed grackles were significantly less glossy than males (Tukey's post hoc, $p<0.05$) and wild-caught males were significantly glossier than museum specimens (see text). Although SY males appear glossier than ASY males, this was not a significant difference (Tukey's post hoc, $p>0.05$). **b** Plumage glossiness (skewness) was significantly positively correlated with sexually selected tail length in both wild and museum-specimen males. There was no significant correlation between glossiness and tail length in females

Table 3 Results of separate ANCOVA for wild male great-tailed grackles with glossiness or each of the five color measures as dependent variables, age class as an independent variable, and tail length, PC1 (body size), PC2 (body mass), and capture date as covariates. Statistically significant results are given in italics, and *df*= 1,6 for all factors

Source	Glossiness (skewness)		Brightness (B1)		Hue (H1)		Hue (H5)		Saturation (S4)		UV/blue chroma	
	F	*p*	*F*	*p*	*F*	*p*	*F*	*p*	*F*	*p*	*F*	*p*
Age	4.29	0.084	0.01	0.91	0.02	0.89	0.01	0.93	1.45	0.27	0.04	0.84
Tail length	8.33	*0.028*	0.78	0.41	0.61	0.46	0.46	0.52	0.01	0.94	1.22	0.31
PC1	1.62	0.25	0.01	0.94	0.001	0.99	0.14	0.72	1.18	0.32	0.13	0.73
PC2	3.19	0.12	8.65	*0.026*	0.54	0.49	3.82	0.098	9.16	*0.023*	3.65	0.10
Date	0.39	0.56	0.15	0.71	0.29	0.61	0.001	0.98	0.43	0.53	0.09	0.78

to body mass (PC2) than tail length (Table 3). However, in separate simple correlation analyses (ignoring body size, PC1; body mass, PC2; sex, and age), tail length is significantly correlated with brightness (B1), saturation (S4), and UV/blue chroma (all $r>0.793$, all $p<0.002$).

We did not detect any blood parasites in our samples, and male plumage glossiness was not significantly correlated with H/L ratio (Pearson's $r=0.061$, $p=0.84$). Female plumage glossiness was not significantly related to tail length, body size, or sampling date ($F_{1,8}\leq 2.23$, $p\geq 0.21$) and did not correlate with any spectral color measurements (Table 2).

Discussion

Here, we make the first attempt to consider the methodological and functional utility of a new color metric—glossiness—in non-human animals. Plumage glossiness, as measured by the skew of log-reflectance distribution, was highly repeatable for all three avian species measured, and these values fell within a range that human observers classify as glossy (Motoyoshi et al. 2007; Sharan et al. 2008). The photographic and computational method we present here is relatively simple; however, a few conditions must be met for proper implementation. First, a suitable camera with the proper settings must be used, including the ability to capture images in an uncompressed format (e.g., TIFF or RAW), to manually set the white balance, and to apply the proper intensity linearization when processing the images. We followed the recommendations of Stevens et al. (2007) when selecting our camera, settings, and implementing the intensity linearization. Second, because glossiness is a specular phenomenon, the orientation of the specimen and lighting conditions can have substantial effects on this measure. We recommend using a standardized light source, mounting the camera at a fixed distance from the specimen, making every effort to orient specimens consistently in the same position, and testing the repeatability of the measurement whenever it is applied to a new system.

We observed considerable inter- and intra-specific variation in the glossiness of all three species we examined. In red-winged blackbirds and Chihuahuan ravens, there was little or no chromatic coloration, and glossiness was not significantly correlated with any measures of color, indicating that chromatic iridescence is not required for the appearance of gloss. The glossiness of great-tailed grackles differed significantly between males and females and was positively correlated with a putatively sexually selected trait, male tail length. When we compared glossiness and conventional color metrics, we found that these different visual metrics captured variation in different aspects of male morphology. Plumage coloration (brightness and saturation) was associated with variation in PC2 (body mass) while glossiness correlated with tail length, suggesting that these components of the visual display could have different signaling functions. These results suggest a role for glossiness in visual signaling, but because glossiness is significantly correlated with coloration (chroma and brightness) in great-tailed grackles, we cannot assess its function independent of color. Subsequent studies should focus on glossy species without a significant chromatic iridescent component to their visual displays (e.g., corvids) to avoid confounding glossiness and coloration in the conventional sense.

Structural, developmental, and environmental factors are known to influence the glossiness of human hair, including cleanliness, the application of oils, and the optical microstructure (Stamm et al. 1977; McMullen and Jachowicz 2003). Consistent with this observation, we found that the plumage of >30-year-old museum specimens of great-tailed grackles were significantly less glossy than the live birds that were actively maintaining their plumage. Therefore, glossiness has the potential to function as an indicator of current condition, perhaps by reflecting plumage self-maintenance (e.g., ruffling, preening); this may include the application of preen oils, which influences plumage appearance and mate choice in other bird species (Zampiga et al. 2004). Preen oil composition and production varies

with diet, age, season, and circulating androgens (Sandilands et al. 2004), protects against feather degradation (Shawkey et al. 2003), and affects the purity of yellow plumage color in great tits (*Parus major*; Surmacki and Nowakowski 2007). Removal and application experiments are now needed to directly assess the effects of both soiling and preen oils on plumage glossiness and its possible use as a signal of quality.

In mammalian hair, bird feathers, and non-biological materials, surface smoothness, and integrity are predictors of glossiness (Stamm et al. 1977; Rasmussen and Dyck 2000; Yonehara et al. 2004, Andersson and Prager 2006). The significant correlation we observed between grackle breast plumage glossiness and the bluish-green iridescent color of those feathers provides some indication of the optical mechanisms that may underlie glossiness. In a broad sample of icterids (including great-tailed grackles; Shawkey et al. 2006) and a study of the satin bowerbird (Doucet et al. 2006), bluish-green iridescent plumage color is associated with the presence of a thin layer of densely packed melanin granules beneath a smooth keratin cortex in the feather barbules. This arrangement creates specular thin-film reflectance and the constructive interference of specific wavelengths of light. However, non-iridescent red-winged blackbirds achieved similar levels of gloss to the great-tailed grackle, but lack a distinct organized outer layer of melanin granules in the feather barbule. This indicates that chromatic iridescence is not required to produce glossiness, and we propose that selection on glossiness may provide an intermediate step in the evolution of complex plumage iridescence, by favoring the evolution of a smooth, specular feather cortex. Once a smooth cortex is present, simple reorganization of the melanin granules within the feather barbules could produce thin-film iridescence. A key next step in understanding the role of glossiness in animal signaling is to use behavioral experiments to test whether and how animals perceive and respond to glossiness independent of coloration.

The natural image statistical approach that we employed here has largely been applied to problems of human and machine vision (reviewed in Geisler 2008). Our study of grackle glossiness is the first application in the context of animal communication. We suggest that these methods are particularly well-suited to studies of animal visual signaling because image statistics provide an opportunity to assess animal visual signals in a holistic way that captures complex interactions and patterns elements within a visual scene that may not be captured by traditional spectrophotometric or physiological modeling studies.

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