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Achieving luster: prenuptial molt pattern predicts iridescent structural coloration in Blue-black Grassquits

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Abstract Colors in feathers are produced by pigment deposition or by nanostructures within barbs or barbules. In the absence of pigments or nanostructures, light is scattered incoherently, producing white coloration. Honest advertisement models predict that ornamental colors evolve if they reliably signal individual properties such as viability, health, or nutritional state. In this study, we tested if (1) iridescent structural and (2) unpigmented plumage coloration signal male quality in the Blue-black Grassquit (*Volatinia jacarina*). During three reproductive seasons, we captured males and measured morphological variables and nuptial plumage coverage, and collected feathers for spectrometry. We found that saturation of the iridescent coloration was positively related to relative molting speed, indicating that males investing more in prenuptial molt also produced more saturated, UV-shifted plumage. Body condition was not related to brightness or saturation of the iridescent plumage, and no male morphological attributes were associated with the white underwing patch coloration or size. Our results suggest that patterns of molt, and possibly feather growth, may affect the organization of optical nanostructures responsible for iridescent coloration, and that the ontogeny of iridescent plumage ornaments must be taken into account in hypotheses concerning honest advertisement of such signals. Thus, coloration in this species may reliably reflect energy allocation to molting, constituting an honest indicator of male quality, life history decisions, or endocrine state. To our knowledge, this is the first report of natural variation in molting pattern being associated with sexually-selected structural color plumage.

Keywords Animal communication · Honest signaling · Molt · Structural coloration · *Volatinia jacarina*

Introduction

Ornamental characteristics in animals have been intensively studied over the past few decades, and growing evidence supports their evolution under sexual selection (Andersson 1994). By combining sexual selection and animal communication theory, several theoretical models have emerged linking ornament expression to its information content, therefore suggesting that ornaments may act as signals of male health, condition and status (Searcy and Nowicki 2005). Honest advertisement models propose that sexual ornaments are costly and can only be sustained by some individuals, thus maintaining signal reliability relative to male quality. Therefore, only higher quality males are able to bear the costs of enhanced expression of an ornament, ensuring that conspecific males and females can dependably extract and rely on information from these signals (Searcy and Nowicki 2005).

The study of birds, which display a dazzling variety of conspicuous songs, colors, and plumage, has greatly

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contributed to our understanding of how sexual selection shapes ornamentation and the communication process. Avian feather colors are produced by two different means: pigments, such as melanins and carotenoids, and feather nanostructures that, owing to their differences in refractive indexes and organization, may interact with incident light and reinforce reflection at certain wavelengths. Furthermore, the lack of pigments or of organized nanostructures scatters light incoherently, producing white color (Prum 2006). Several studies have shown that individuals that bear brighter and more chromatically-defined colors may be older (Inouye et al. 2001), in better nutritional condition during molt (Hill and Montgomerie 1994), or carry lower parasite loads (Hõrak et al. 2004). Also, correlational and experimental studies have indicated that the information content of such color patches may influence dominance hierarchies in male–male interactions (Wolfenbarger 1999) and/or female mate choice (Marchetti 1998), with brighter and more colorful males obtaining competitive or mating advantages.

Most of the studies conducted on avian coloration in relation to sexual selection and communication have focused on carotenoid pigments, given that the costs of allocating carotenoids to plumage ornaments are considerably better understood than melanin-pigmented and structurally colored ornaments (McGraw 2006). It is well known that carotenoids have antioxidant properties and must be obtained through the diet, whereas evidence on the information content and role of structural coloration in intraspecific interactions is still ambiguous and poorly understood. While some studies have found a positive association between structural color properties and male quality characteristics, such as nutritional condition and parasite load (Keyser and Hill 1999; McGraw et al. 2002; Hill et al. 2005), others have failed to find such a relationship (Perrier et al. 2002; Balenger et al. 2007), or have found unexpected ones, i.e. negative relationship between parasite load and both coloration and body condition in Blue-black Grassquits (*Volatinia jacarina*), but no association between coloration and body condition (Costa and Macedo 2005), and no assortative pairing nor relation between color and body condition in Azure-winged Magpies (*Cyanopica cyanus*), but negative relation between both parents' plumage brightness and chance of nest predation (Avilés et al. 2008). Additionally, evidence of the use of this information in intraspecific communication is still equivocal (Siefferman and Hill 2005; Korsten et al. 2007; Liu et al. 2007, 2009 Loyau et al. 2007). Knowledge of proximate and developmental mechanisms of inter-individual variation of color properties, essential to the elaboration of condition-dependency hypotheses for such traits, has only recently emerged (Shawkey et al. 2003; Doucet et al. 2006; Prum et al. 2009). Particularly in the case of glossy dark colors, usually produced by a single thin layer of keratin over a layer of melanin granules, disturbance during feather development has been suggested to affect the production and organization of color-producing structures (Prum 2006), therefore proposing a basis for condition-dependent expression of these ornaments. However, since virtually nothing is known about the mechanisms that regulate the organization of these nanostructures and how this occurs during feather ontogeny (Ghiradella and Butler 2009), it is impossible to propose a clear hypothesis concerning the mechanisms regulating condition-dependence of iridescent coloration.

The Blue-black Grassquit exhibits behavioral and ecological characteristics that make it a valuable model organism to investigate the role of structural colors in social communication. This species occupies a large geographic range that extends from Mexico to most of South America (Sick 1997). During the breeding season, Blue-black Grassquits migrate from the Amazon region into central Brazil, where we conducted our study. During this period, males undergo a prenuptial molt from a drab female-like plumage to an iridescent blue-black nuptial plumage with contrasting white underwing patches. Also during reproduction, males defend small, clustered territories and repeatedly execute a vertical leaping display (Almeida and Macedo 2001), in which both the color properties of the blue-black plumage and the white patch are exhibited. Such a conspicuous visual exhibition, together with the evidence of extra-pair fertilization rates of 50% of nestlings (Carvalho et al. 2006), point to the possibility that males are subject to intense sexual selection pressures, and highlights the possible role of coloration in this process.

Probably the first evidence of condition-dependence of iridescent structural coloration in a bird came from a study of the Blue-black Grassquit (Doucet 2002). Results from that study indicated that, among several measures of male quality, only feather growth bars (intercalating light and dark bands representing feather growth during the day and the night, respectively; Grubb 1989, 1991) in flight feathers were correlated to color characteristics, suggesting that color properties reflect male nutritional condition during molt, and thus the allocation of resources to feather growth. However, in our study population, birds do not molt flight feathers during the prenuptial molt: no bird captured during the three study years presented symmetric flight feather molting on both wings (R.M. and R.H.M., personal observation). Our observations thus do not support a direct link between growth bars and nutritional condition during the prenuptial molt.

The objective of our study is to clarify the information content of the ornamental coloration of Blue-black Grassquits. We tested the hypothesis that variation in structural color properties of the blue-black plumage and of the white underwing patch (the latter which has not been considered previously) are related to characteristics that reflect individual quality of males during the prenuptial molt. To test this hypothesis, we appraised male quality using indexes of prenuptial molting (which has been suggested to reflect parasite load; Costa and Macedo 2005; Aguilar et al. 2008) and body condition, and examined how these relate to color properties.

Methods

Study area and field methods

Birds were captured in a 2.6-ha abandoned orchard within a preserved Cerrado (Neotropical grassland) matrix at Fazenda Água Limpa (15°56′S, 47°56′W), a farm property of the Universidade de Brasilia, Brazil. Birds were captured using mist-nets placed daily in the study site during the breeding seasons of 2005/2006, 2006/2007 and 2007/2008. Only males that were captured for the first time, and which later established territories in our study site, were included in our analyses.

Blue-black Grassquits migrate from the Amazon to central Brazil around September, remaining in mixed-sex feeding flocks for about 3 months. Throughout this period, most males are undergoing molt to the nuptial plumage, and a few can only be distinguished from females due to slightly darker flight feathers. Prenuptial molt is characterized by a full-body (including wing and underwing coverts) molt to the iridescent plumage with white underwing patches. Only around November/December do males begin defending territories and breeding, which lasts until April/May (Almeida and Macedo 2001). Most males are fully molted to the nuptial plumage at this point, although males can defend territories, display and reproduce with only partially molted plumage (Doucet 2002; R.M. and R.H.M., personal observation). Therefore, we focused our capture effort between September and January during all three breeding seasons, but exact dates varied depending on bird arrival in the study area and onset of breeding.

Captured birds were banded with numbered metal bands (Brazilian bird-banding institution—CEMAVE/IBAMA) and with a unique combination of three colored plastic bands. We used digital sliding calipers to take morphometric measurements of the left tarsus, and a Pesola® scale to take bird mass. We estimated the coverage of nuptial plumage by placing a small transparent disc, equally divided into eight sections, over four body parts (head, back, rump and chest) and counting the sections that superimposed blue-black plumage. A mean proportion value was obtained by averaging the measurements from all four body parts and dividing by the number of sections in the disc. For the measurement of the area of the white underwing patch, we traced an outline of the wing and the patch on an acetate sheet placed over the fully open wing (Costa and Macedo 2005). Afterwards, we digitized the sheets at 300 dpi and calculated patch area with ImageJ software (v.1.40; Rasband 1997–2004) using a standardized threshold procedure. The average of the underwing patch area divided by total wing area for both wings was used as a measure of patch size, controlling for wing size.

For color measurements, we plucked three feathers each from the head, back and chest, and two feathers from each of both white underwing patches. If individuals were under partial molt to the nuptial plumage when captured, feathers clearly identifiable as nuptial plumage were collected to ensure consistency of the comparison of color properties. We taped the feathers to a black card and wrapped it in foil paper, after which it was kept under dry and stable temperature conditions in the laboratory until the analyses took place.

Spectrometry

For spectral measurements, feathers taken from each body area were taped to a black velvet substrate in an overlaid manner to simulate their arrangement on the bird's body. Feathers from both white underwing patches were placed together for a single measurement. We conducted reflectance measurements with an Ocean Optics USB4000 portable spectrometer attached to a PX-2 pulsed xenon light source (Ocean Optics, Dunedin, Florida). All measurements were taken relative to a WS-1-SS white source (Ocean Optics) and to the substrate (dark reference). Optic fibers were held using a block sheath to exclude ambient light and maintain the probes at a constant distance (6 mm) from the feather surface.

Due to the iridescent properties of Blue-black Grassquit coverts, we used two separate probes, one attached to the light source and one to the spectrometer, specularly placed at 45° from the plane perpendicular to the feather surface. This measurement geometry was used since it provided the most repeatable and saturated spectra, while still highly correlated to other measurement geometries such as under normal light incidence (Maia et al. 2009), offering a more reliable extraction of colorimetric components. Barbule tilting in relation to the barb axis can also influence color measurements (Osorio and Ham 2002), which would explain why the non-iridescent white underwing feathers also had higher reflectance peaks under this geometry, as has been recently demonstrated for other species' non-iridescent feather patches (Santos et al. 2007). For similar reasons, we used measurements from feathers extracted from the back of individuals to characterize their plumage color, since these feathers were larger and more densely-packed with barbules, thus providing more reliable measurements (Quesada and Senar 2006) that were still correlated to measurements from the head and the chest.

We used SpectraSuite software (Ocean Optics) to measure and record 50 sequential spectra from the feathers. Five measurements were taken from each sample, lifting the sheath from the feather surface between each measurement to ensure that a different portion was being measured and that overall plumage properties were captured. The average of these five spectra, interpolated to a step width of 1 nm between 300 and 700 nm, was used to characterize individual color (Fig. 1a).

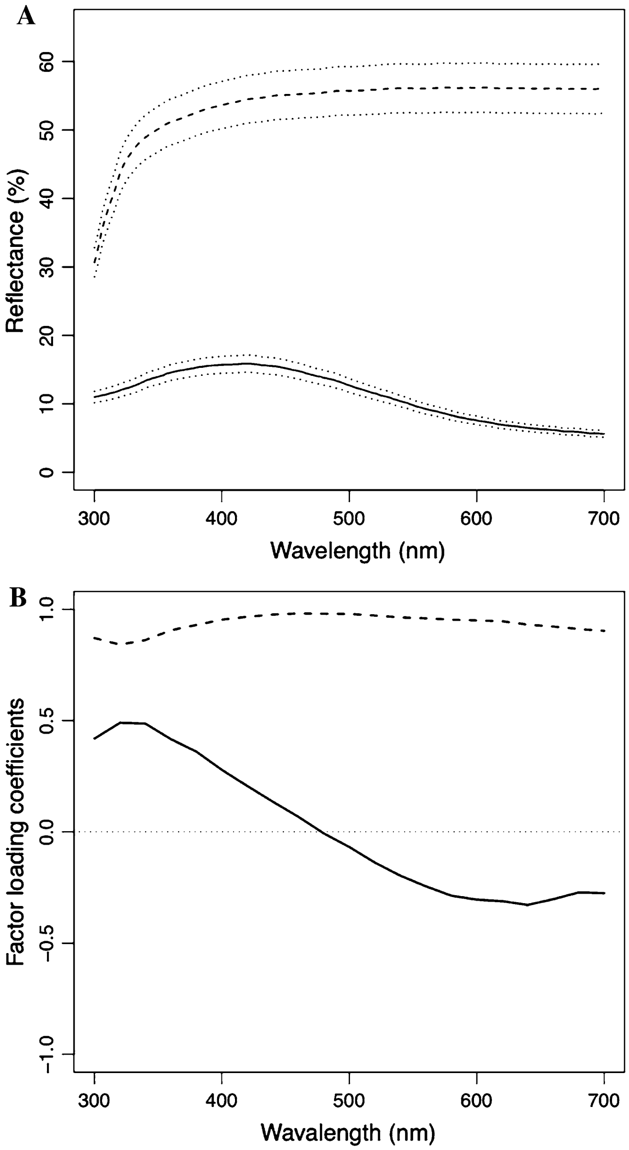

Fig. 1 **a** Mean reflectance spectra of male Blue-black Grassquit (*Volatinia jacarina*) iridescent feathers (*solid line*, $n = 52$) and white underwing patch feathers (*dashed line*, $n = 56$) measured at 45°. *Dotted lines* indicate 95% confidence intervals. **b** Factor loading coefficients for PC1 (*dashed line*) and PC2 (*solid line*) of the iridescent coloration in relation to wavelength

Color properties

To avoid multiple testing with correlated measurements, we conducted a principal component analysis (PCA on correlation matrices, no factor rotation) on data from individual average reflectance curves of the iridescent plumage (Hunt et al. 1998; Montgomerie 2006). We averaged reflectance values in 20-nm intervals across the measured spectra, using the resulting 20 values as variables and individual measurements as observations. Individual scores for the first two components, which together accounted for 96.58% of the total variance, were then used as variables throughout the analyses.

For ease of interpretation of the obtained PC variables, spectral reflectance characteristics for the iridescent nuptial plumage were summarized into estimates of brightness, saturation and hue (Montgomerie 2006), which were then correlated to both PC variables. Average brightness (R_{avg}) and peak reflectance intensity ($R_{\max}$) were taken to characterize brightness. The wavelength at peak reflectance was used to define hue, and two variables were calculated for spectral saturation:contrast ($R_{\lambda\max}/R_{\lambda\min}$) and UV chroma ($R_{300\text{–}400\text{nm}}/R_{300\text{–}700\text{nm}}$). These variables and their relation to PC scores are summarized in Table 1. PC1 displayed positive and high factor loadings at all wavelengths, and therefore was taken to represent overall brightness. PC2, on the other hand, had positive factor loadings for short wavelengths and negative loadings for long wavelengths, and therefore was interpreted as a measure of spectral purity, or saturation, of plumage color, after controlling for brightness variation (Fig. 1b). Due to the spectral properties of the white underwing patch, the only color variables considered were brightness and UV chroma (Doucet et al. 2005).

Measures of male quality

One male quality measure was estimated by a body condition index, calculated by dividing each individual's mass by its tarsus length. This measurement has previously been shown to indicate overall male health status both under field and laboratory conditions (Costa and Macedo 2005; Aguilar et al. 2008; Santos et al. 2009). Thus, high values of this index are expected for well-nourished males, while relatively low values indicate males lighter than expected for their size.

Relative molting speed can also be considered a measure of male quality, since it should reflect the capacity an individual has to invest in the growth of new feathers. Regression models based on the molting scores of birds are commonly used to estimate beginning, end, and speed of molt (Ginn and Melville 1983). Although individual birds are only captured once, these models have allowed researchers to obtain such estimates for the expected population values. Thus, we assume that regression parameters represent expected values

Table 1 Descriptive color variables for the iridescent nuptial plumage and white underwing patch of male Blue-black Grassquits (*Volatinia jacarina*), and Pearson correlation coefficients (*r*) for the relationship between iridescent color variables and principle component scores (bold values: $P < 0.001$ after Bonferroni correction)

Variable	Nuptial iridescent plumage ($n = 52$)	White underwing patch ($n = 56$)	*r*	
			PC1	PC2
Average brightness (%)	11.22 ± 0.43	53.71 ± 7.11	**0.99**	0.08
Peak reflectance intensity (%)	16.18 ± 0.63		**0.96**	0.25
Contrast	3.07 ± 0.09		−0.18	**0.67**
Hue (nm)	409.65 ± 3.70		0.10	**−0.68**
UV chroma	30.71 ± 0.48	22.43 ± 2.97	0.01	**0.91**
Variance explained (%)			87.80	08.78

for mean molting scores over time, and therefore deviations from these parameters—the regression residuals—represent individual variation over the predicted values for a given moment in time. Hence, we calculated a relative molting speed index by using the residuals of the linear model of percent of nuptial plumage coverage (arcsine-transformed) as the response variable, capture date as the explanatory variable, and capture year as a covariate (date: $\beta = 0.71$, $F_{1,50} = 46.36$, $P < 0.001$; year: $F_{2,50} = 0.11$, $P = 0.89$; model: $R^2 = 52.91\%$, $F_{3,50} = 18.73$, $P < 0.001$). Since few males were captured multiple times throughout the breeding season, we cannot discern if this index expresses earlier onset of molting or faster molting. Either way, the index can be taken to reflect a male's investment in prenuptial molt, since high values correspond to individuals in a relatively more advanced stage of molting than expected when controlling for capture date and year, while low values indicate individuals with less nuptial plumage than expected for that period (Fig. 2).

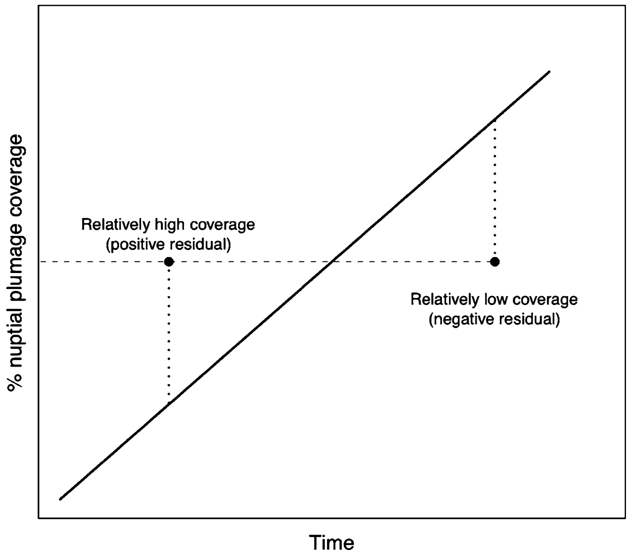

Fig. 2 Schematic representation of how the molting index was obtained. The *regression line* represents the average (expected) values of nuptial plumage coverage as a function of time. Note that, for the same values of coverage percentage, the relative molting score can be either positive or negative depending on when the value was observed

Statistical analysis

We conducted separate linear regressions considering the nuptial color principal components and the white patch size and color variables as response variables, male quality indexes as the explanatory variables, and year as a covariate. Backward stepwise procedures were used to obtain the most parsimonious model, and explanatory variables were excluded from the final model when they did not approach significance ($P > 0.1$). Since year was included for adequate partitioning of variance and not as a predictor, it was included even when nonsignificant. Finally, to understand the relationship between nutritional condition (as represented by the relative body mass index) and investment in molting, we tested for the relationship between these two variables.

Test assumptions were verified by checking the distribution of response variables and diagnostic plots of the linear models (residuals vs fitted values, normal probability plot and Cook's distance; Maindonald and Braun 2007), and outliers were removed when identified. All analyses were conducted in R statistical software (v.2.9.2; R Development Core Team 2009). Sample sizes may differ between model groups since it was not possible to collect all variables from every individual, but models with the same response variable are based on the same data frame. Unless otherwise noted, all results are presented as mean ± SE.

Results

We captured 30 males during the 2005/2006 breeding season, 18 during 2006/2007, and 8 during 2007/2008, for a total of 56 analyzed individuals. Over the considered visual geometry, reflectance of the nuptial plumage peaked on short wavelengths (Fig. 1a), with 14 males (25%)

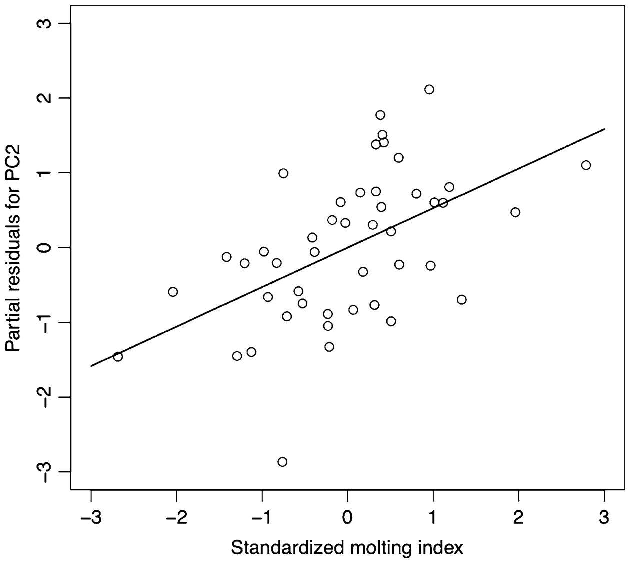

Fig. 3 Relationship between the partial residuals (values for the response variable after accounting for the variation due to year) of PC2 (representing spectral purity, with higher values indicating greater contrast and UV chroma, and shorter-wavelength hue) and molting index in male Blue-black Grassquits

presenting hue in UV wavelengths, 37 (67%) in violet-blue wavelengths, and four (8%) over 600 nm (and therefore classified as "brown" individuals). Since we were interested in the signaling properties of nuptial plumage, we did not include the latter in our analyses. Reflectance of the white underwing patch presented a marked decrease in intensity over short wavelengths (Fig. 1a).

Nuptial plumage brightness (PC1) was not associated with any measure of male quality (all variables $P > 0.05$). Color saturation (PC2) of the blue-black plumage, on the other hand, was positively associated with the molting index (molting index: $\beta = 0.53 \pm 0.13$, $F_{1,42} = 15.48$, $P < 0.001$; year: $F_{2,42} = 0.53$, $P = 0.59$; model: $R^2 = 27.06\%$, $F_{3,42} = 5.20$, $P < 0.01$; Fig. 3). Therefore, males that invest in molting to the nuptial plumage faster and/or earlier do so to more saturated, UV-shifted color plumage. Neither plumage brightness nor color saturation was correlated with the body condition index.

Brightness, UV reflectance and white underwing patch area were not associated with either body condition or molting indexes (all models $P > 0.05$). Also, molting index was not associated with the body condition index (body condition: $\beta = 0.19 \pm 0.18$, $F_{1,42} = 1.11$, $P = 0.29$; year: $F_{2,42} = 0.31$, $P = 0.74$; model: $F_{3,42} = 0.92$, $P = 0.44$).

Discussion

In this study, we show that color properties of the iridescent blue-black nuptial plumage of male Blue-black Grassquits reflect male attributes during the prenuptial molt, which occurs just prior to breeding. Males in a relatively more advanced stage of molting at a given time displayed more saturated, UV-shifted coloration than individuals in earlier molting stages during the same time period. Thus, males that allocate relatively more resources into molting produce considerably more chromatically defined nuptial plumage. Contrary to our expectations, however, no color properties were associated with male body condition; also, underwing patch size and color do not seem to reflect any attribute of male quality.

In a previous study of the same species, a positive relationship between feather growth (band width of retrices, used as a measure of nutritional condition during molt) and color score was found for attributes measured after the breeding season (Doucet 2002). Also, a relationship between nuptial plumage coverage and body condition (as measured by feather growth) was found, though the former was not associated with color attributes (Doucet 2002). However, the use of flight feather growth rates to estimate body condition during molting (Grubb 1989) may be problematic since, in our study population, as in several other species that alternate between a nuptial and an eclipse plumage, Blue-black Grassquits do not molt flight and covert feathers (which characterize the iridescent nuptial plumage of males) during the same period (Humphrey and Parkes 1959). It is probable that flight and covert feather molting are regulated by hormonal mechanisms (Collis and Borgia 1993; Dawson and Sharp 1998; Peters et al. 2000; Badyaev and Vleck 2007). Under this condition, flight and covert feather growth rates may be directly associated with color properties even in the absence of an effect of nutritional condition, thus compromising the use of growth bars as an indicator of nutritional condition in the case of structurally colored ornaments.

It is important to note that variation in the molting index can be the result of three different, non-mutually exclusive processes: (1) onset of molting, (2) number of simultaneously molting feathers, and (3) feather growth speed. Based on our data, we cannot discern whether individuals with more saturated coloration initiated prenuptial molt earlier, shed more feathers at a time, or molted at a faster rate. Nonetheless, we believe that these processes reflect differential energy allocation to the costly activity of molting. Early molting can have elevated costs associated with thermoregulation and, in the case of bright plumage, predation risk, but may also result in greater benefits because of early territory acquisition and initiation of visual displays (Mulder and Magrath 1994; Cockburn et al. 2008). Further, molting is an energetically demanding activity (Ginn and Melville 1983), and overlapping it with other activities can incur fitness costs (Svensson and Nilsen 1997; Hemborg et al. 1998). Hence, individuals that undergo early prenuptial molting should either be in an initial superior condition or better capable of handling such costs, and may be selected to signal this capacity through plumage properties (such as color). As for the case of molting speed, studies have shown that feather growth rate can vary drastically among individuals and between molts of the same individual (De la Hera et al. 2009). Additionally, food deprivation can reduce growth rate of individual feathers, even without affecting overall molting rate and duration (Swaddle and Witter 1997), therefore suggesting that differences in molting speed may also be associated with individual feather growth rates. However, though these three scenarios can potentially link molting speed to its cost, more information is necessary to understand the link between molt, feather development, and coloration.

Birds commonly undergo molt after breeding (and prior to migration, when applicable; Gill 2007). In this case, late molting birds may reduce the effects of this delay by molting faster, therefore reducing the negative effects of late molting (but see Dawson et al. 2000). In a prenuptial molt scenario, however, the fitness consequences of delaying or slowing down molt can be very different, especially when individuals are molting to a sexually selected ornamental plumage. In Superb Fairy-wrens (*Malurus cyaneus*), for example, undergoing a prenuptial molt right after migration and just prior to breeding can be extremely costly (Mulder and Magrath 1994), and has been shown to be a trait selected for by females when pursuing extra-pair copulations (Dunn and Cockburn 1999). Therefore, allocating energy to an ornament in such a critical moment may pose different costs to low- and high-quality individuals, enforcing signal honesty despite the lack of a direct link to nutritional condition. In House Finches (*Carpodacus mexicanus*), for example, a straightforward link between condition and ornament elaboration occurs only in juveniles and nonbreeding adults, whereas breeding adults, despite molting later, faster, and in worse body condition, may produce equally elaborate feather ornaments (red bib: Badyaev and Duckworth 2003; Badyaev and Vleck 2007).

The effect of molting speed on color attributes has so far received little attention in the literature. By regulating daylength, Serra and collaborators (2007) have shown that Rock Sparrows (*Petronia petronia*) exposed to shorter daylength (which induced faster molting) produce sexually selected yellow patches that were smaller and less chromatically defined. Similarly, fast-molting Blue Tits (*Cyanistes caeruleus*) under similar experimental conditions produced less saturated yellow feathers (Ferns and Hinsley 2008). These studies corroborate field data showing that younger Blue Tits and Great Tits (*Parus major*), which molt faster than adults, had less saturated yellow flanks (Ferns and Hinsley 2008). However, in both studies, the ornamental color analyzed was a pigment-derived color. In such cases, carotenoid pigments are deposited in the feather barbs during feather development, so that faster growing feathers receive less pigment (Badyaev and Landeen 2007). Only one study has investigated the effect of molt speed on structural colors, suggesting that fast molting reduces brightness and UV chroma of the non-iridescent crown of Blue Tits (Griggio et al. 2009). Though differences in feather nanostructure were not assessed in that study, the authors suggest that molt speed may affect the regularity of light-scattering nanostructures, therefore influencing the produced color (Griggio et al. 2009).

Experimental manipulation to determine the effects of molt speed upon iridescent colors has yet to be conducted. Nonetheless, knowledge of the physical mechanisms of iridescent structural color production may provide further insight into our findings. The iridescent coloration of male Blue-black Grassquits results from a thin keratin layer and a single underlying layer of melanin granules, in which the thickness of the keratin layer, the number of melanin granules and the optical properties of this pigment influence the resulting color (Maia et al. 2009). Saturation and hue of color produced by single-layer films are determined by the optical distance light must penetrate these layered structures, i.e. layer thickness and refractive index (Kinoshita et al. 2008). Brightness, on the other hand, is not influenced by single-layer structural organization (given a constant refractive index). This has been demonstrated in the Satin Bowerbird (*Ptilonorhynchus violaceus*), for example, where hue and saturation were shown to be positively associated with the keratin cortex thickness and melanin density, respectively, but no structural predictors were found for brightness (Doucet et al. 2006).

Given the similarity between the color-producing structures in Satin Bowerbirds and Blue-black Grassquits, the relationship found in our study suggests that molting speed influences the structural organization of barbules, since chromatic properties, but not brightness, were associated with the molting index. Thus, investment in molting would probably result in greater spectral purity by influencing nanostructural organization in feather barbules, without affecting overall feather properties at a larger scale (such as number of barbules or barbule tilting, which could influence preceived brightness; Osorio and Ham 2002; Doucet et al. 2006). Further knowledge on the ontogeny of iridescent feathers is thus crucial for the understanding of condition-dependence mechanisms of this type of ornament.

We did not find a relationship between body condition and investment in prenuptial molt. Parasite load has been previously shown to affect the amount of nuptial plumage of male Blue-black Grassquits, despite not being associated with body condition (Costa and Macedo 2005). Our results support these findings, suggesting that an individual's ability to invest in ornamental plumage may be restricted by factors affecting molt directly, and not only indirectly through nutritional condition. Such factors may include, in addition to aforementioned parasite effects, genetic and hormonal attributes, age, or a trade-off between current and future expectations of reproduction (Badyaev and Qvarnström 2002).

Neither color properties nor size of the white underwing patch were related to male quality in this study. Though white color patches (Hanssen et al. 2006; McGlothlin et al. 2007), their size (Török et al. 2003) and their shape (Ferns and Hinsley 2004) have been shown to reflect individual quality in other species, the proximate mechanisms regulating condition-dependence of white feathers remain largely unknown (Shawkey et al. 2006). The underwing patches in the Blue-black Grassquit may have evolved through Fisherian processes (as suggested by Costa and Macedo 2005), in which a correlation between female preference and male ornamentation could drive the exaggeration of characteristics such as size and color properties in the absence of any information content of such ornaments. Another possibility is that, since these white patches are only visible when grassquits are displaying or flying, they may have evolved to increase contrast with the dark iridescent nuptial plumage. In fact, recent comparative analyses among bird species have suggested a relationship between dark-and-white contrast and behavioral displays (Galván 2008), increasing the conspicuousness of individuals and their color attributes during leap displays. If white underwing patches indeed function as amplifiers to the signal in the iridescent plumage, the direction of the association between male quality and patch characteristics may be dependent on male quality itself (Galván and Sanz 2008).

In conclusion, our data suggest that iridescent coloration in Blue-black Grassquit nuptial plumage reflects male investment in prenuptial molt. This finding is in accordance with the concept of honest advertisement: the interindividual, differential costs of plumage production will only allow those males that have the necessary resources to invest in ornament development. In the case of Blue-black Grassquits, this means that either faster or earlier molting is essential to the production of highly saturated plumage, peaking in the UV spectrum. To our knowledge, this study is the first attempt to discern between the effects of body condition and natural variation in molting patterns in the expression of structurally based feather color properties. Current studies still have not elucidated how molting and feather growth affect coloration, and our results suggest that future hypotheses linking these traits will be crucial, particularly in the case of structurally-colored ornaments. White plumage, on the other hand, showed no relation to male quality, suggesting its role as a contrast amplifier during leaping displays. Further studies should clarify the differential costs of iridescent coloration in birds by investigating proximal mechanisms of both color production and prenuptial molt, and attempt to link these to life history correlates of investment in ornamentation.

Zusammenfassung

Wie das Gefieder Glanz erhält: Das Muster der Pränuptialmauser sagt die irisierende Strukturfärbung bei Jakarinifinken *Volatinia jacarina* vorher

Federfarben werden durch Pigmentablagerung oder Nanostrukturen innerhalb der Federäste oder -strahlen erzeugt. In Abwesenheit von Pigmenten oder Nanostrukturen wird das Licht inkohärent gestreut, was eine weiße Färbung bewirkt. Aufrichtige Signal-Modelle sagen vorher, dass Schmuckfarben evolvieren, wenn sie individuelle Eigenschaften, wie zum Beispiel Lebensfähigkeit, Gesundheit oder Ernährungszustand, verlässlich anzeigen. In dieser Studie haben wir untersucht, ob (a) irisierende Strukturfärbung und (b) unpigmentierte Gefiederfärbung bei Jakarinifinken (*Volatinia jacarina*) die Männchenqualität signalisieren. Über drei Brutsaisons haben wir Männchen gefangen und morphologische Variablen sowie Balzkleidbedeckung gemessen und Federn für Spektrometrie gesammelt. Wir fanden, dass die Sättigung der irisierenden Färbung mit der relativen Mausergeschwindigkeit in positiver Beziehung stand, was darauf hindeutet, dass Männchen, die stärker in die Pränuptialmauser investierten, auch ein stärker gesättigtes, UV-reflektierendes Gefieder produzierten. Die Körperkondition hing nicht mit Glanz oder Sättigung des irisierenden Gefieders zusammen, und kein morphologisches Merkmal der Männchen war mit der Färbung oder Größe des weißen Unterflügelflecks assoziiert. Unsere Ergebnisse deuten darauf hin, dass die Muster der Mauser und möglicherweise das Federwachstum den Aufbau der optischen Nanostrukturen, die für die irisierende Färbung verantwortlich sind, beeinflussen dürften. Außerdem muss die Ontogenese der irisierenden Federornamente für Hypothesen, welche das aufrichtige Zeigen solcher Signale betreffen, berücksichtigt werden. Daher könnte die Färbung bei dieser Art verlässlich die Energiezuteilung für die Mauser widerspiegeln und somit einen ehrlichen Indikator für die Qualität, life history-Entscheidungen oder endokrinen Zustand der Männchen darstellen. Unseres Wissens ist dies der erste Nachweis einer Assoziation zwischen natürlicher Variation im Mausermuster und sexuell selektiertem Gefieder, das durch Strukturfarben gefärbt ist.

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