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FEMALE MATE CHOICE IN RELATION TO STRUCTURAL PLUMAGE COLORATION IN BLUE GROSBEAKS

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Abstract. Plumage blueness in Blue Grosbeaks (*Passerina caerulea*) is related to nutritional condition during molt, and bluer males hold larger territories with more food resources. We tested the hypothesis that females use male plumage brightness as a criterion in choosing mates. In a mate-choice aviary, we presented females with a choice between males whose feathers were either brightened with blue marker or dulled with black marker. Treatment resulted in an increase in the plumage intensity (reflectance at peak wavelength) of blue-treated males and a decrease in the intensity of black-treated males within the natural range of variation, while the wavelength of peak reflection (hue) remained unchanged. During mate-choice trials, females consistently preferred particular males, but choice was random with respect to treatment. Thus, the intensity of blue coloration of male Blue Grosbeaks appears not to function as a criterion in female mate choice.

Key words: Blue Grosbeak, female choice, Passerina caerulea*, structural color.*

Elección de Pareja en Hembras de *Passerina caerulea* con Relación a la Coloración Estructural del Plumaje

Resumen. Estudios previos han demostrado que la coloración azul del plumaje de *Passerina caerulea* está relacionada con la condición nutricional durante la muda y que los machos de coloración más azul mantienen territorios más grandes y con mayores fuentes de alimento. En el presente estudio, examinamos la hipótesis de que las hembras usan el brillo del plumaje como criterio para la selección de parejas. En una arena de selección de parejas, presentamos a las hembras con la opción de elegir entre machos de plumaje coloreado artificialmente con marcador azul o machos de plumaje artificialmente coloreado con marcador negro. El tratamiento del plumaje produjo cambios en la intensidad de color de tal forma que incrementamos la intensidad de color en los machos tratados con marcador azul y redujimos la intensidad de aquellos tratados con marcador negro. La longitud de onda del pico de reflección (tono) no cambió. Durante las pruebas de selección de pareja, las hembras mostraron una preferencia consistente por ciertos machos, pero la elección fue aleatoria con respecto al tratamiento. Por lo tanto, la intensidad de coloración azul de *P. caerulea* no parece servir como criterio selectivo en la elección de pareja.

Models that explain the evolution and maintenance of secondary sexual traits in terms of female choice predict that females should prefer traits that most reliably reflect male quality (Zahavi 1975, Hamilton and Zuk 1982, Kodric-Brown and Brown 1984, Grafen 1990, Johnstone and Grafen 1993, Andersson 1994). Because ornamental plumage has been found to be costly in birds (Hill 1991, 1994, 1996, Hill and Montgomerie 1994), it may provide an excellent index of male viability or genetic quality (Andersson 1986, 1994). Ornamental feather coloration is produced by pigments or feather microstructure (Fox 1976), with melanin and carotenoid pigments accounting for most pigment-based coloration. Structural coloration is the result of feather microstructure that differentially reflects and scatters wavelengths of light (Fox 1976). Blues and bright greens are, in most cases, the result of precise arrangements of feather microstructures found on the barbs and barbules of feathers (Dyck 1971, 1978, 1987, Andersson 1999, Prum et al. 1999). Until recently, most research on the function and evolution of plumage coloration has focused on melanin- and carotenoid-based displays.

Structural coloration often extends into the ultraviolet (UV) portion of the electromagnetic spectrum (320–400 nm, Finger and Burkhardt 1994), so variation in structural coloration can be invisible to the human eye (Bennett and Cuthill 1994, Bennett et al. 1994). Behavioral evidence suggests that birds respond to UV signals and that both males and females use UV color in mate-choice decisions (Bennett et al. 1996, Andersson and Amundsen 1997, Hunt et al. 1997, 1999, Andersson et al. 1998). Almost all tests of mate choice for structural plumage coloration have focused on the effects of UV light, and have manipulated plumage coloration by placing females behind viewing windows opaque to UV light (Bennett et al. 1996, 1997, Andersson and Amundsen 1997, Hunt et al. 1997, 1999). These studies clearly show that both males and females avoid potential mates that entirely lack UV coloration in their plumage, but they provide little insight into how natural variation in structural coloration

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affects individual attractiveness (Bennett et al. 1997). To more precisely test whether structural plumage coloration functions in female choice, it is necessary to manipulate the plumage in such a way as to mimic natural variation.

Blue Grosbeaks (*Passerina caerulea*) are a good species for such a study because the blue plumage of males is structurally based, reflects maximally in the UV range, and may function as a signal of male quality (Keyser and Hill 2000). Plumage brightness in Blue Grosbeaks may be condition dependent (Keyser and Hill 1999) and is correlated with territory size and territory quality (Keyser and Hill 2000). Furthermore, there is substantial variation among males in plumage blueness, a composite measure that incorporates peak wavelength, intensity, and percentage of blue plumage (Keyser and Hill 1999), such that there is the potential for expression of plumage blueness to serve as a signal to conspecifics.

The purpose of this study was to experimentally test the role of blue plumage coloration in female mate choice in the Blue Grosbeak. We manipulated plumage coloration to make one set of males above average in plumage brightness and a second set of males below average in plumage brightness. We then tested the response to these manipulated males by female Blue Grosbeaks in breeding condition in outdoor aviaries.

METHODS

STUDY AREA

This study was conducted in an outdoor aviary located on the campus of Auburn University in Lee County, Alabama, during May and June, 1999. Blue Grosbeaks were trapped in Lee County either in live traps baited with seed or in mist nests using a playback and model in April and May, 1999. Males and females were captured at different locations separated by at least 20 km. We deemed males to be in breeding condition by appearance of cloacal protuberance and breeding behavior such as territory defense and singing. Females were trapped as they arrived on their breeding grounds, which is two or three weeks before they begin breeding activities. Thus, we deemed females to be in prime breeding condition. Individuals received aluminum U.S. Fish and Wildlife Service bands. Upon capture, mass, tarsus length, and natural wing chord were measured for all individuals. For males, we recorded detailed plumage information and collected contour feathers by plucking 8–12 feathers from the breast and rump for spectral analysis. Feathers were stored in paper envelopes until they were analyzed.

Females were housed in one group in a large outdoor cage (5.5 × 3.7 × 1.5 m) while males were housed in smaller outdoor cages (3.7 × 1.5 × 2.4 m) in flocks of 4–6 that were visually, but not acoustically, isolated from each other and from females. All birds received sunflower seed, millet, mealworms (*Tenebrio tenebrio*) and water *ad libitum.* Sulfadimethoxine (1 g L^{-1}) was added to water to prevent coccidial infection. We allowed males and females to acclimate to captivity for at least two weeks before they were used in trials.

PLUMAGE COLOR QUANTIFICATION

Spectral peak and intensity are accurate and repeatable measures of feather color (Endler 1990). Intensity is measured as percent reflectance at particular wavelengths along a color spectrum, measured in nanometers. Spectral peak, or the wavelength of peak reflectance in a single-peaked spectrum, is likely to correlate with plumage hue as perceived by birds, while percent reflectance at the peak wavelength corresponds to plumage brightness.

Yearling Blue Grosbeaks have a variable proportion of brown feathers in their plumage. For this study we used only adult males with greater than 90% of their bodies covered with blue plumage. To quantify the spectral components of blue plumage, we taped feathers onto white cards in an overlapping fashion, mimicking how feathers lie on a bird's body. The resulting small patch (approximately 1 cm^2) of blue feathers was analyzed following methods of Keyser and Hill (1999). We used an Ocean Optics S2000 fiberoptic spectrometer (range 250–880 nm; Ocean Optics, Inc., Dunedin, Florida) with a 200-micron fiberoptic probe at a 90° angle to the feather surface. The probe is equipped with a 6-mm-long cylindrical metal sheath to exclude ambient light. The end of the sheath rests on the feather sample, and the probe illuminates a 2-mm-diameter circular area with a deuterium bulb (ultraviolet light source) and a tungsten-halogen bulb (visible light source; Analytical Instrument Systems, Inc., Flemington, New Jersey). Reflectance spectra were generated relative to a Spectralon™ white standard (Labsphere, Inc., North Sutton, New Hampshire). Spectra were obtained via a spectral acquisition software package (Ocean Optics, Inc.). For each spectral scan, 20 spectra were taken sequentially and then averaged, thereby reducing the effect of electrical noise on spectra. For each feather sample, five scans were taken and averaged. Between each scan, the probe was lifted and replaced in a different arbitrary place on the feather sample. Wavelength of peak reflectance and intensity at peak reflectance were obtained from spectral data for each feather sample.

MORPHOLOGICAL MANIPULATION

We evaluated female choice by presenting each female with a dyad of males. We selected males for a dyad so that they were as close as possible to each other in terms of plumage coloration (peak wavelength, intensity, and percent blue plumage) as well as in body size based on their overall rank in the captive males used in the trials. There were no significant differences between members of each treatment in body size and plumage color when compared with an independent *t*-test (Table 1). We randomly determined the treatment for each male in a dyad by flipping a coin. On the day preceding the mate-choice trial, we used blue marker on the plumage of one male (increased intensity) and black marker on the plumage of the other male (decreased intensity). We used nontoxic blue, Tria™ art markers (Letraset Ltd., Ashton, UK) and nontoxic black, Sharpie® markers (Sanford, Bellwood, Illinois). For all applications, we covered all body and head plumage as evenly as possible. Application of markers on plumage did not affect feather quality: the marker

TABLE 1. Plumage and body size measures (means ± SD) for male Blue Grosbeaks before and after plumage manipulation. Measures are compared using independent t-tests (2-tailed for pretreatment, 1-tailed for post-treatment). Plumage data were not available for three post-trial males, and post-trial weights were not taken. Pretreatment males were similar in plumage and size, and the treatment had the intended effect of increasing the difference in plumage intensity without changing peak wavelength. Body size was indexed as tarsus length/body mass.

	Pretreatment			Post-treatment		
	Reduced intensity (n = 6)	Increased intensity (n = 6)	t	Reduced intensity (n = 4)	Increased intensity (n = 5)	t
Breast						
Peak wavelength (nm)	404.6 ± 9.5	402.8 ± 8.7	0.3	402.2 ± 11.3	401.9 ± 10.2	0
Intensity (%)[a]	35 ± 9	38 ± 8	–0.7	38 ± 5	49 ± 9	–2.2*
Rump						
Peak wavelength (nm)	413.4 ± 12.1	411.6 ± 11.2	0.3	405.8 ± 11.2	403.3 ± 12.2	–0.3
Intensity (%)[a]	44 ± 18	42 ± 10	0.2	36 ± 9	54 ± 6	–3.6**
Body size index (mm g^{-1})	0.8 ± 0.0	0.8 ± 0.0	–0.4			

[a] Percent reflectance at peak wavelength.
* $P < 0.05$; ** $P < 0.01$.

dried quickly and feathers were not matted or clumped after treatment.

MATE-CHOICE TRIALS

Mate-choice trials took place in a large outdoor mate-choice aviary, which is separated into five sections, four sections for males and one section for a female (Fig. 1). The aviary is constructed with plywood and wire mesh such that the upper portion of the entire aviary (outer walls and roof) are exposed to natural light. Male sections are separated by plywood, and male and female sections are separated by wire mesh on the top half and plywood on the bottom half. The female portion spans the entire length of the aviary, allowing for visual and acoustic but not physical contact with all male sections. The female section is divided lengthwise by a plywood half-wall extending from the center of the ceiling halfway to the floor. The purpose of the wall is to require females to choose to interact with males by flying underneath the wall, reducing the possibility that interactions with males are the result of random activities.

After treatment, males were moved to the mate-choice aviary and placed into the two end sections, separated by the two empty middle sections and their plywood walls. A randomly chosen female was introduced into the mate-choice aviary and placed into the female section. Males and females were given 24–48 hr to acclimate before we collected data. The mate-choice aviary was videotaped for the first 2 hr after dawn on 2 consecutive days for later analysis. Females were used only once, there were a total of six dyads of males, and five dyads were used twice. When reusing dyads, we switched males from left to right cage to control for position. We considered each female as an independent data point for the analysis and thus we used the total number of females as our sample size.

Trials began when the female left the neutral area of the mate-choice aviary and entered the portion that interfaced with male sections. For each minute over the next hour, we tallied the number of times a female visited each male. Each time a female perched in front of a male or each time she performed a circular ‘‘invitation flight’’ in front of male cages we tallied a visit. For each minute, we determined a winner by counting the number of visits in front of each male. If there were an equal number of visits to each male we considered that minute to be a tie. We did not include ties or time spent in the neutral area in the statistical analysis.

STATISTICAL ANALYSES

For statistical analyses we used a paired t-test on the arcsine-transformed proportions of minutes won by each male and we used a chi-square test to compare the observed values of chosen males with expected values of chosen males if choice was random with respect to the treatment. We used independent t-tests to compare body size and coloration measurements of males before and after treatment. Untransformed data are reported as means ± SD. We used a power analysis on transformed mate-choice data to determine the power of our experiment to detect an effect of the treatment.

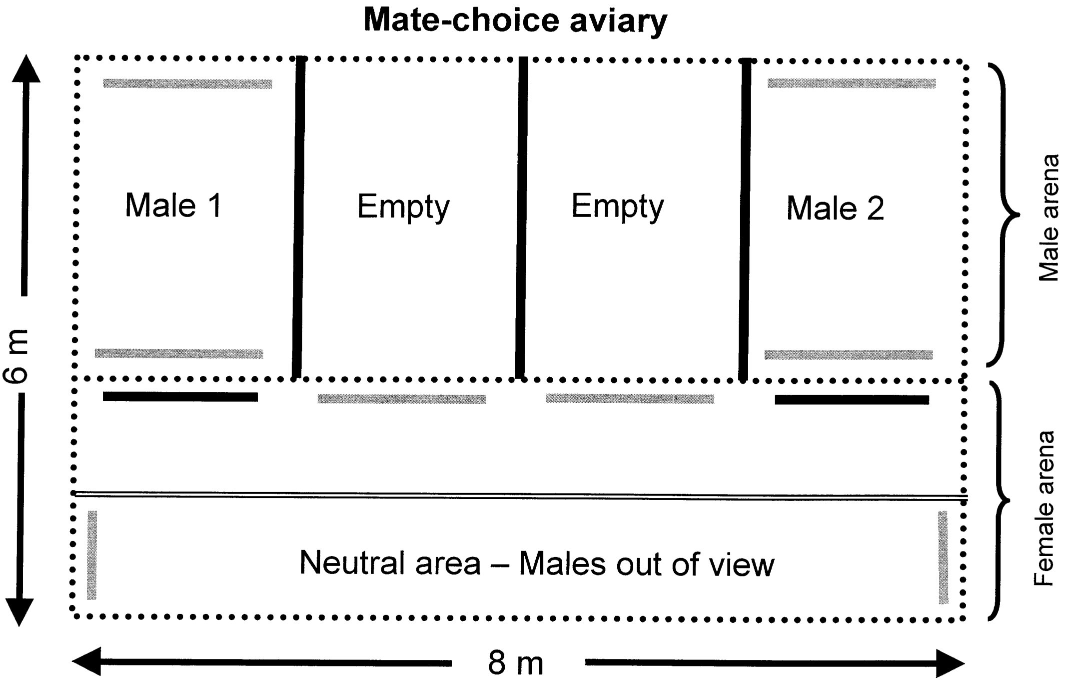

FIGURE 1. Overhead schematic of outdoor mate-choice aviary used for Blue Grosbeak female choice experiment. Outer walls (dotted lines) are plywood on the bottom half and wire mesh on the top half; separations between male cages (solid lines) are plywood. The roof is wire mesh to allow natural light. A plywood half-wall running from the ceiling halfway to the floor (double line) separates the female area into a "neutral area" that contains perches, food, and water, and visually separates females from males. For a female to display mate choice, she must fly underneath the wall, where she can view and hear males in all cages. Males and females are physically separated by wire/plywood walls. Perches (short, black or gray solid bars) are available in male and female cages and in the neutral area. Females using perches shown in black are considered to have visited the male in that cage. Visits to perches shown in gray are considered unrelated to male choice.

RESULTS

Based on our visual assessment, the marker treatment had no or minimal effect on the shade of the plumage of male Blue Grosbeaks. Birds colored with a black marker still appeared bright blue. Spectral analysis indicated that the treatment significantly changed color intensity but not hue (Fig. 2, Table 1) primarily in the UV portion of the spectrum. The changes in intensity did not exceed the natural range of variation in this population (Keyser and Hill 1999).

Males were very active in the presence of females, vocalizing and otherwise behaving normally. Females were generally interested in males and often perched in front of them. Females showed no preference for the side of the arena (right side = 0.56 ± 0.32; left side = 0.44 ± 0.32, $t_{10} = -0.7$; $P = 0.5$, $n = 11$). Seven of 11 females showed a consistent mate choice between trials, choosing the same male on the second day that they did on the first day. Of the females whose choice changed on consecutive days, three females showed a very strong preference on the second day of the trial (a single male won 100% of minutes). Of the 11 females tested, five showed an overall preference for the male with reduced intensity (black treatment), and six showed an overall preference for the male with increased intensity (blue treatment; $\chi^2_{10} = 0$, $P = 0.5$). A paired *t*-test also showed no difference in the total proportion of minutes won for black-treatment males (0.44 ± 0.32) versus the total proportion of minutes won for blue-treatment males (0.56 ± 0.32, $t_{10} = 0.8$, $P = 0.5$, $n = 11$).

DISCUSSION

Blue Grosbeaks vary greatly in plumage intensity, and this intensity is correlated with condition (Keyser and Hill 1999). Thus we hypothesized that plumage intensity functions in female choice in Blue Grosbeaks. Our result that female Blue Grosbeaks chose males irrespective of plumage intensity does not support our hypothesis. While other tests have shown that females avoid males whose UV coloration has been completely removed (Bennett et al. 1996, Andersson and Amundsen 1997, Hunt et al. 1997, 1999), this is one of the first tests of female choice that approximates the natural range of variation in a structurally based coloration. Although our results are based on a small sample

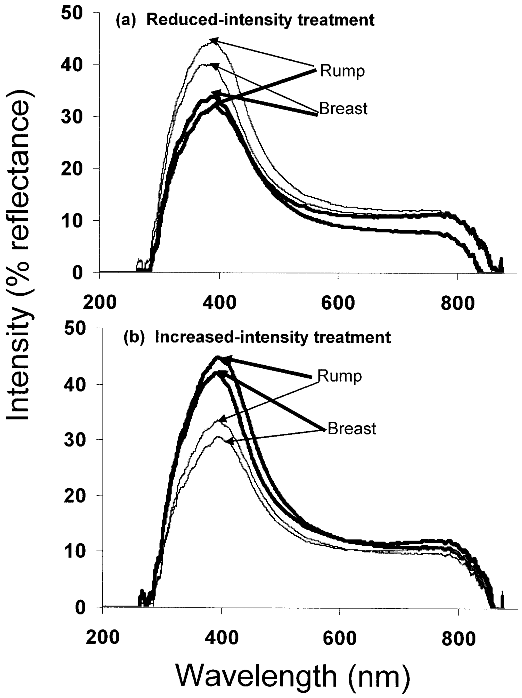

FIGURE 2. Reflectance spectra showing the effect of plumage-intensity (a) reduction (black marker) or (b) augmentation (blue marker) on the rump and breast feathers of two representative male Blue Grosbeaks. On both spectra, the thin gray line is the spectrum before marker treatment, and the bold black line is the spectrum after treatment. The natural range of variation in intensity at the peak wavelength for Blue Grosbeak breast feathers is 14–53%, and for rump feathers is 13–55% ($n = 78$).

size (power = 0.20), given the variation we observed, it would be necessary to test 110 females to detect an effect of our treatment with a power of 0.90. Thus, we conclude that variation in plumage intensity has a negligible effect on female mate choice in Blue Grosbeaks.

A possible confound of our experimental design could be that by letting females acclimate to the mate-choice aviary before mate-choice trials began, we missed the period of actual mate choice. However, observations during trials suggest that females were still soliciting males for attention throughout the experiment. During trials, test females interacted with males using close-contact vocalizations and flight displays. Test males also were very active during trials, attracting females with vigorous flight displays, close-contact vocalizations, and song. Most females made a consistent choice between observation periods. The few females that switched preferences showed a stronger preference on the second day, suggesting that some females required longer to make that decision. Although how female Blue Grosbeaks choose males in the wild is unknown, evidence from other species (Great Reed Warbler [*Acrocephalus arundinaceus*]) suggests that females may take up to three days to decide in the wild (Bensch and Hasselquist 1992). Thus, by allowing females to take several days to sample males, we feel our experimental design mimicked how females may actually choose mates in wild populations. Since all females made a clear mate choice during trials, perhaps other aspects of male behavior influenced female choice. For instance, males sang and displayed throughout trials. Unfortunately, we were unable to measure these behaviors during trials.

Female Blue Grosbeaks may choose mates indirectly by assessing territory quality rather than by assessing male quality using plumage. It is primarily the female who feeds nestlings (Keyser and Hill 2000), so females may benefit more from choosing good territories than from choosing mates themselves. Indirect mate choice by females for territory quality generally sets up conditions for intrasexual competition (Wiley and Poston 1996) and a male's reproductive success may depend on his ability to compete with other males for a superior territory. Plumage intensity in Blue Grosbeaks is positively correlated with body size, territory size, and territory quality but not paternal feeding rate (Keyser and Hill 2000), which is consistent with an intrasexual function of plumage intensity. Thus, an alternative hypothesis for the function of plumage intensity in male Blue Grosbeaks is that it is used in intrasexual interactions to acquire superior territories, which is necessary to gain access to females. Future studies should focus on the role of male-male competition in the evolution of plumage coloration in Blue Grosbeaks.

The aviary facility was approved by the Auburn University Institutional Animal Care and Use Committee (IACUC). Animals were handled according to protocols approved by the IACUC (PRN# 0008-R-2010). We would like to thank Alex Badyaev, Anne Dervan, Renèe Duckworth, Jeremy Hyman, Amber Keyser, Paul Nolan, and Lynn Seifferman for helpful comments on this manuscript. We would especially like to thank Lynn Seifferman for her help with capturing adults and transcribing videotapes. We give special thanks to David S. Dobkin and two anonymous reviewers for suggestions and comments that greatly improved this manuscript.

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