EVIDENCE FOR SEXUAL SELECTION ON STRUCTURAL PLUMAGE COLORATION IN FEMALE EASTERN BLUEBIRDS (*SIALIA SIALIS*)

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Abstract.—Although the function of ornamental traits in males has been the focus of intensive research for decades, expression of such traits in females has received much less study. Eastern bluebirds (*Sialia sialis*) display structurally based ultraviolet/blue and melanin-based chestnut plumage, and in males this plumage coloration is related to both reproductive success and competitive ability. Compared to males, female bluebirds show a subdued expression of blue and chestnut ornamental coloration, and we used a combination of an aviary nutritional-stress experiment and four years of field data to test the hypothesis that coloration functions as a signal of female quality. First, we tested the effect of food intake on expression of structural and melanin coloration in female eastern bluebirds to determine whether structural or melanin coloration are condition-dependent traits. Females that were given ad libitum access to food displayed more ornamented structural coloration than females on a food-restricted diet, but there was no effect of the experiment on melanin ornamentation. Second, we used field data to assess whether female ornamentation correlated with measures of mate quality and parental effort. The structural coloration of females predicted first egg date, maternal provisioning rates, and measures of reproductive success. These data indicate that structural coloration is dependent on nutritional condition and suggest that sexual selection is acting on structurally based plumage coloration in female eastern bluebirds.

Key words.—Condition-dependent traits, melanin color, nonrandom mating, sexual selection, structural color.

In many species of animals, males have more elaborate ornamental traits than females and this has been attributed to stronger sexual selection on males compared to females (Darwin 1871). At the extreme, Lande (1980, 1987) argued that ornaments in females serve no function and exist only as a correlated response to selection for those traits in males. However, in socially monogamous species with biparental care, there should be mutual mate choice because males as well as females should benefit from discriminating between potential mates (Ligon 1999). Characteristics that indicate a high quality male partner might also identify a high quality female partner, in which case female ornamentation might serve a function similar to that of male ornamentation (Johnstone et al. 1996). Furthermore, when breeding resources are scarce, competition within both sexes can be intense and could select for display traits that function as signals of resource holding potential in contests for access to breeding resources or mates (Johnstone et al. 1996). Indicator models of sexual selection propose that the expression of sexually selected traits reliably signal individual condition (Andersson 1994). Because only exceptionally fit individuals in a population achieve maximum expression of such traits, these traits can act as honest signals of an individual's phenotypic or genetic quality (Zahavi 1975; Hamilton and Zuk 1982; Kodric-Brown and Brown 1984). These models predict that individuals that display more exaggerated traits compete better for mates and thus experience higher reproductive success.

One manifestation of selection for the same ornamental trait in males and females is assortative mating—pairing of individuals of similar phenotypes. Assortative mating by ornamental traits might indicate that males and females exert mutual mate choice and have similar preferences for phenotypes (Johnstone et al. 1996) or that such traits function

in intrasexual competition for territories by both sexes (Creighton 2001). Studies have demonstrated assortative mating by ornamentation (e.g., Hill 1993; MacDougall and Montgomerie 2003), by age (Warkentin et al. 1992), and by body size (Bonduriansky 2001).

A large body of literature suggests that sexual selection is responsible for much of the diversity in ornamental plumage coloration in male birds (reviewed in Hill and McGraw 2005). Moreover, although females often appear less colorful than males, there is still a plethora of diversity in the coloration in females and this suggests that it may function in sexual selection. Plumage color of both males and females is most commonly derived from one of three distinct mechanisms: carotenoid pigments, melanin pigments, or feather microstructure (Fox and Vevers 1960; Hill and McGraw 2005). Colors that result from these different mechanisms may have different costs associated with their production, and hence signal different aspects of individual condition. Although there is extensive empirical evidence that expression of ornamental plumage coloration is condition dependent in males of many taxa (Andersson 1994; Hill 2006), few studies have experimentally tested the effects of specific environmental variables on expression of female plumage coloration. However, the experimental studies to date suggest that patterns of condition dependency in female plumage traits mirror patterns of condition dependency in the equivalent male traits (reviewed in Amundsen and Pärn 2006).

For instance, carotenoids are generally regarded as the most clearly condition-dependent form of plumage coloration in males (Hill 2002). Just as in males, in female house finches (*Carpodacus mexicanus*) color expression is affected by access to pigments (Hill 1993), exposure to parasites (Hill 2002), and food restriction (Hill 2002). Melanin coloration,

on the other hand, seems less affected by environmental conditions than carotenoid coloration (Hill and Brawner 1998; Roulin et al. 1998; McGraw and Hill 2000), but it is possible that access to scarce amino acids (Jawor and Breitwisch 2003) or rare minerals (McGraw 2003) might limit expression.

Variation in noniridescent structurally based coloration is largely a function of the anatomy the spongy layer of feather barbs (reviewed in Prum 1999). To date, experimental tests of the production costs associated with structural coloration have focused only on iridescent plumage in males. In male brown-headed cowbirds (*Molothrus ater*), nutritional stress reduced the brightness of structurally based body color (McGraw et al. 2002), and in the wild turkey (*Meleagris gallopavo*), males infected with coccidial parasites grew less colorful iridescent feathers than males that were not infected (Hill et al. 2005).

We studied the function and proximate control of structural and melanin coloration of female eastern bluebirds (*Sialia sialis*), a species in which female coloration might serve as a signal and be subject to sexual selection. Males exhibit noniridescent, ultraviolet/blue, structurally based plumage over their back, head, wings, and tail, and chestnut melanin-based plumage on the breast. Females show the same basic color pattern as males, but the structural coloration appears duller and more blue-green whereas the breast coloration is paler. Blue structural and chestnut melanin coloration are sexually selected traits in male bluebirds. Males that exhibit more ornamented structural and melanin plumage coloration nest earlier, are better parents, and enjoy higher reproductive success (Siefferman and Hill 2003). Bluebirds are obligate secondary cavity nesters and both males and females compete vigorously against same-sex conspecifics for nest sites (Gowaty and Wagner 1988). Moreover, removal experiments have shown that males and females are usually replaced within 24 h of departure from their territory, indicating the existence of a large number of unmated adult bluebirds of both sexes (L. Siefferman, unpubl. data). Structural plumage coloration appears to influence male-male contests for nest sites (Siefferman and Hill 2005) and ornaments that are used in aggressive signaling should function in both sexes (Ligon 1999). Extrapair paternity is common in bluebirds (accounting for as many as 40% of offspring; Gowaty and Karlin 1984) and conspecific brood parasitism also occurs (up to 10% of offspring; Gowaty and Karlin 1984), increasing the potential variance in reproductive success of both males and females. Further, because both sexes care for young (Gowaty and Plissner 1998) both sexes could increase their reproductive success if they made good mate-choice decisions (Johnstone et al. 1996). Thus, although one might expect that selection for reliable signals of quality would be stronger in males than in females, individuals could benefit from assessing honest indictors of female quality.

In this study, we tested the hypothesis that plumage coloration in eastern bluebirds is a sexually selected honest indicator of female quality. First, we used a laboratory experiment to determine the effect of nutritional constraints during molt on the expression of melanin-based and structurally based ornamental plumage coloration of female eastern bluebirds. Second, we collected four years of field data to investigate whether pairs mated assortatively by color and to

assess relationships between female plumage coloration and date of first nest, provisioning rates to offspring, condition of offspring at fledging, and number of offspring fledged.

MATERIALS AND METHODS

Aviary Methods

During May 2002, we used mist nets to capture 14 adult female eastern bluebirds that were in their second breeding season in Macon County, Alabama (32°33′N, 82° 28′W). Birds were captured at their nest boxes during early spring. We housed birds indoors in individual cages (0.5m × 0.5m × 0.5m) at an aviary on the campus of Auburn University. Each cage contained wooden dowels as perches and steel grates covering the cage bottoms so feces and discarded food would not collect on the cage floor. Natural day/night cycles were maintained via light timers and windows emitting natural light near cages. Prior to and during the experiment, captive females from both experimental and control groups were given a diet of meal worms supplemented with Bluebirds Forever bluebird food (Bluejay, CA; ground suet, grains, peanuts and raisins). Within the 14 cages of bluebirds, we randomly assigned eight to the food-deprivation group and six as controls. The prebasic molt occurs from late August through October for eastern bluebirds (Gowaty and Plissner 1998), thus we ran food-deprivation experiments from July15 until all birds completed molt by November 1. We removed the food dishes from experimental groups for randomly selected six-hour periods of daylight on two of every three days during molt. We used this randomized design to prevent birds from anticipating periods of food removal and ingesting large quantifies of food prior to deprivation periods. Although control groups had access to the diet throughout the study, we opened cages and raised and lowered dishes to provide similar levels of disturbance. We quantified body mass and the melanin and structural plumage coloration of the breasts and rumps of female eastern bluebirds at two times: (1) before birds began molting in captivity (prior to the experiment in July) and (2) upon molt completion (after completion of the experiment in November). We did not measure tail coloration in this experiment because the tails were damaged and abraded by contact with the floor and walls of the cages. This experiment follows protocols that have revealed the condition dependency of carotenoid and structural plumage coloration (Hill 2000, 2002; McGraw et al. 2002).

Field Methods

We monitored a population of eastern bluebirds in Lee County, Alabama (32°35′N, 82° 28′W) for four breeding seasons (1999–2002). We captured males and females at nest sites in early spring and marked them with unique combinations of three color bands and one numbered metal band. We estimated the age of all newly banded birds as either yearling (in their first year of life) or in the second or subsequent year based on the shape of the 10th primary feather (Pitts 1985). For a subset of birds that were banded as nestlings on our field site, we knew the exact age. For each bird, we measured mass, tarsus length, and plumage coloration, and we used the residuals of a mass to tarsus regression as

an index of body condition (Jakob et al. 1996). One hundred and fifty nest boxes were checked at least once every three days throughout the breeding season to document the date at which the first egg was laid.

We measured the rates at which parents provisioned nestlings using 8-mm video cameras mounted to tripods at a distance of three meters from the nest. We recorded parental activity at the nest for four continuous hours beginning between 0600 and 0700, when chicks were seven days of age. We quantified the female and male provisioning rates as the number of provisioning trips per minute per nestling.

At age 14 days posthatch, we measured the mass of nestlings. From hatching until about 11 days after hatching, nestling mass increases rapidly, but by age 13 days, mass begins to asymptote (Pinkowski 1975). Hence mass at day 14 is an accurate estimate of fledging mass. Bluebirds in Alabama can rear up to three successful broods per season. We measured annual reproductive success as the total number of offspring fledged in the season and offspring condition as the mean nestling mass at age 14 days.

Color Analysis

We measured plumage color using an Ocean Optics S2000 spectrometer (range 250–880nm: Dunedin, FL.) from plucked feathers (see detailed methods in Siefferman and Hill 2003). We took five spectral readings for each of three plumage regions of every individual captured in the wild (breast, rump, and tail).

We summarized reflectance data by calculating three standard descriptors of reflectance spectra: brightness, chroma, and hue. Brightness (total amount of light reflected by the feather) was calculated as the summed reflectance from 300 to 700 nm. We calculated hue and chroma differently for blue and chestnut plumage because of the inherent reflectance properties of the two colors. For the rump and tail feathers, UV chroma, a measure of spectral purity, was calculated as the proportion of the total reflectance (R300–700) that is in the UV part of the spectrum (R300–400). For breast feathers, red chroma was calculated as the proportion of the total reflectance (R300–700) that is in the red part of the spectrum (R575–700). Hue is the principal color reflected by the feather. For structural coloration, hue was calculated as the wavelength at peak reflectance. Because hue of breast (calculated as the wavelength of maximum slope) was nearly invariant among birds, we do include breast hue.

Statistical Analyses

Because plumage characteristics, body condition, first egg date, and nestling condition differed significantly by year (ANOVA: all $P < 0.001$), we standardized all data to a mean of zero and standard deviation of one. For all analyses, if females bred more than one year, we randomly chose one year of data for that female to include in the analysis. We tested for normality using Sharpiro-Wilk tests, using parametric tests when data were normally distributed and nonparametric tests when assumptions of normality were violated. We used SPSS (ver. 11.5; SPSS, Inc., Chicago, IL) software to analyze the data and all statistical tests were two tailed. When appropriate, we adjusted our alpha level to account for multiple comparisons (breast, rump, and tail) of correlated data using the Dubey/Armitage-Parmar correction (Sankoh et al. 1997).

Describing Plumage Color Using Principal Components Analysis

We performed separate principal components analyses (PCA) on measures of breast, rump, and tail coloration. We used this analysis for two reasons. First, the plumage characteristics (brightness, chroma, hue) for each body region were correlated, and second, this analysis allowed us to reduce the number of color variables into a more manageable number (from nine plumage variables to three). Melanin breast coloration (brightness and chroma) reduced to one principal component that explained 72% of the variation among these variables (brightness loading, 0.85; red chroma loading, −0.85). An individual with a high positive PC1 score had lighter plumage and less red chroma and was, therefore, *less* ornamented. The first principal component for structural rump coloration explained 67% of the variation among these variables and received strong loadings from brightness, UV chroma, and hue (0.66, 0.94, −0.82, respectively). An individual with a high positive PC1 score for rump color was more ornamented with brighter, greater UV chroma, and with a more left-shifted hue. The first principal component for tail coloration, which explained 67% of the variation among these variables, received strong loadings from brightness, UV chroma, and hue (loadings; 0.66, 0.89, −0.87). An individual with a high positive PC1 score for tail color was more ornamented, displaying brighter, greater UV chroma, and with a more left-shifted hue.

RESULTS

Nutritional Stress and Female Plumage Color

There was a significant negative effect of nutritional stress during molt on structurally-based UV-blue plumage (PC1); females that were food-stressed grew rump plumage with hues that were more right shifted, less UV chromatic, and duller than females in the control group (Table 1, Fig. 1). This relationship remained significant after we used Dubey/Armitage-Parmar correction to adjust our alpha level to $P \leq 0.028$ to account for multiples comparisons of plumage traits (mean correlation of breast and rump color = 0.16). However, there was no significant effect of nutritional stress on melanin-based chestnut plumage (PC1; Table 1). There also was no significant effect of the nutritional stress on female body mass; in fact, females that were food stressed tended to lose less body mass than females in the control group (Table 1).

Dimorphism

Male eastern bluebirds display significantly more ornamented breast coloration (PC1; $Z = 5.2$, $P < 0.001$, $n = 220$) than females; males had breast feathers with lower total reflectance (darker) and greater red chroma than females (Fig. 2). Male eastern bluebirds displayed significantly more ornamented rump coloration (PC1; $Z = 13.1$, $P < 0.001$, $n = 228$) than females; the rump feathers of males were brighter, expressed greater UV chroma, and had more UV-shifted hues

TABLE 1. Mean change in plumage coloration and body mass for female eastern bluebirds maintained either with ad libitum food or subjected to periods of fasting during molt. Values are means ± SE (sample sizes) and 95% confidence intervals; comparisons are two-tailed Wilcoxon signed rank tests.

Proportional change in trait	Food-stressed	Control	Lower CI	Upper CI	Z	P
Melanin breast	-0.03 ± 0.72 (6)	0.50 ± 0.10 (6)	-1.29	2.35	-0.3	0.81
Structural rump	1.69 ± 0.12 (8)	2.16 ± 0.13 (6)	0.07	0.84	2.32	0.02
Body mass	0.81 ± 0.39 (8)	-0.01 ± 0.48 (6)	-2.14	-2.14	0.51	0.28

than females (Fig. 2). Males also displayed significantly more ornamented tail coloration (PC1; $Z = 11.3$, $P < 0.001$, $n = 218$) than females; the tails of males were brighter, had greater UV chroma, and had more UV-shifted hues than females. These relationships remained significant after we used a Dubey/Armitage-Parmar correction to adjust our alpha level to ($P \leq 0.022$) to account for testing the influence of sex on three different body regions (mean correlation of breast, rump, and tail color = 0.27).

Assortative Mating

Female age was significantly positively related to the age of their mate ($rs = 0.49$, $P < 0.001$, $n = 233$, Fig 3). If we included only the 21 pairs of birds for which we knew the exact age (birds were banded as nestlings) the pattern was even stronger ($rs = 0.65$, $P < 0.001$, $n = 21$). Within pairs, there were no significant correlations between the sexes with respect to tarsus length, body mass, or body condition ($rs = 0.11$, $P = 0.10$, $n = 233$; $r = -0.10$, $P = 0.13$, $n = 231$; $r = -0.12$, $P = 0.07$, $n = 230$). Moreover, we found no evidence for assortative mating by measures of melanin breast coloration (PC1: $rs = -0.07$, $P = 0.32$, $n = 220$) or by measures of structurally-based rump coloration (PC1: $rs = 0.04$, $P = 0.55$, $n = 228$). Finally, there was a tendency for female tail coloration to be related to male tail coloration (PC1: $rs = 0.15$, $P = 0.03$, $n = 218$), this relationship was not significant after we used a Dubey/Armitage-Parmar correction to adjust our alpha level (to $P \leq 0.022$) to correct

for measuring the plumage coloration of three body regions (mean correlation of breast, rump, and tail color = 0.27).

Relationships among Plumage Measures

We used linear regressions to investigate relationships among measures of plumage coloration of the breasts, rumps, and tails of female eastern bluebirds. Females with less ornamented breasts (higher PC value; lighter in color) expressed more ornamented rump coloration ($r^2 = 0.03$, $F^1_{226} = 6.0$, $P = 0.01$). Breast and tail coloration were significantly related (PC1; $r^2 = 0.11$, $F^1_{223} = 27.1$, $P < 0.001$; Fig 4), such that females displaying less ornamented breasts had more ornamented structural coloration. Measures of structural coloration were also significantly related ($r^2 = 0.10$, $F^1_{224} = 23.8$, $P < 0.001$) such that females displaying more ornamented rumps had more ornamented tails.

Female Ornamentation and Age

To determine whether body condition or plumage characteristics differed according to female age class, we used multivariate analysis of variance (MANOVA). Females of different age classes did not differ significantly in body condition or coloration (Table 2). When we included only the 42 birds for which we knew exact age (birds were banded as
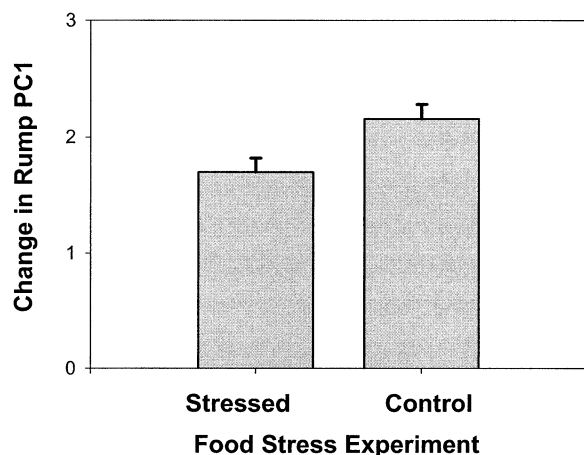

FIG. 1. Mean change in structural coloration (PC1) plumage coloration (postmolt–premolt) for female eastern bluebirds maintained either with ad libitum food or subjected to periods of fasting during molt.
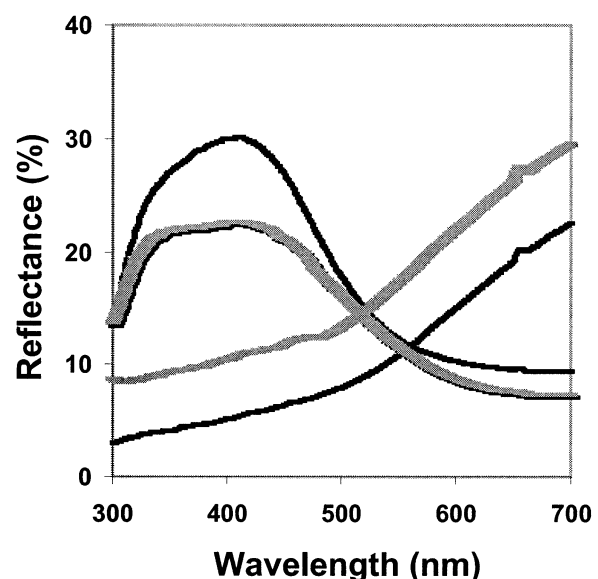

FIG. 2. Reflectance spectra of male eastern bluebird structural and melanin plumage (thin line) and female eastern bluebird structural and melanin plumage (thick line).

FIG. 3. Relationships between male and female age within pairs of eastern bluebirds, the variation in point sizes represent overlapping points (148, 23, 21, 14, 7, 7, 7, 4, and 2 points).
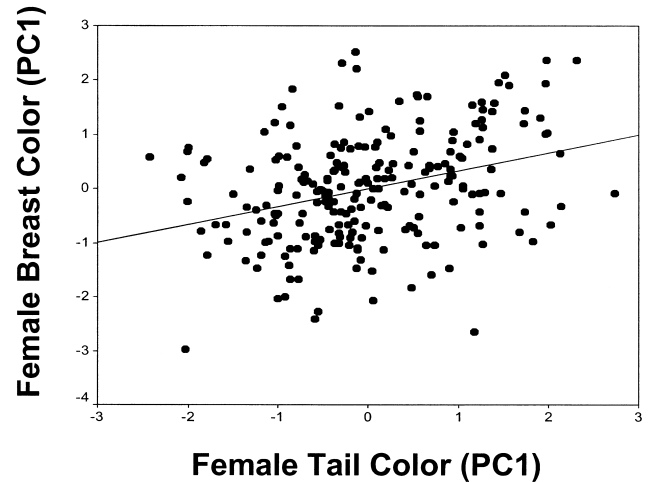

FIG. 4. Relationship between female tail coloration (standardized for year effects, PC1, lower score corresponds to most ornamented plumage) and female breast coloration (standardized for year effects, PC1, higher score corresponds to most ornamented plumage) of eastern bluebirds.

nestlings) we also found no difference in plumage coloration among age groups.

Female Ornamentation and Body Condition

We used linear regressions to investigate relationships between plumage coloration and the body condition index measured in early spring. Body condition was not a significant predictor of breast coloration ($r^2 = 0.01$, $F^1_{227} = 2.44$, $P = 0.12$), rump coloration ($r^2 = 0.001$, $F^1_{225} = 0.22$, $P = 0.64$), or tail coloration ($r^2 = 0.01$, $F^1_{222} = 1.75$, $P = 0.19$).

Female Ornamentation, First Egg Date, Provisioning Rates, and Reproductive Success

To determine the value of plumage color, body condition, and age in predicting the first egg date of female eastern bluebirds, we constructed a backward stepwise multiple regression model. In this model, we included breast, rump, and tail color scores, body condition, and age as potential predictor variables. Tail coloration (Fig. 5), female age, and body condition were significant predictors of first egg date. Thus, females with more colorful tails, older females, and females in better body condition initiated egg laying earlier in the season ($r^2 = 0.08$, $F^3_{216} = 5.6$, $P < 0.001$; Table 3).

To determine which variables influenced the provisioning rates of female eastern bluebirds, we constructed another backward stepwise multiple regression model. In this analysis, we included scores for both male and female breast, rump, and tail color scores, body condition, maternal age, and first egg date as potential predictor variables. Female

rump coloration, male rump coloration, female body condition, and female age were significant predictors of female feeding rate. Females with more colorful rumps, females mated to males with more colorful rumps, females in better body condition, and younger females provisioned offspring at higher rates ($r^2 = 0.08$, $F^4_{159} = 3.0$, $P = 0.02$; Table 3). To investigate whether females might preferentially mate with males that are ''good parents,'' we compared female traits with male provisioning rates. We constructed backward stepwise multiple regression models, including female breast, rump, and tail color, body condition, maternal age, and first egg date as potential predictor variables. Female age was a significant predictor of male provisioning rates, such that younger females mated with males that provisioned chicks more often ($r^2 = 0.02$, $F^1_{54} = 3.8$, $P = 0.05$; Table 3).

To determine which variables influenced the condition of fledglings, we constructed a backward stepwise multiple regression model. In this analysis, we included scores for both male and female breast, rump, and tail color scores, body condition, maternal age, first egg date, and total parental feeding rates as potential predictor variables. Female tail coloration and parental feeding rates were significant predictors of fledgling condition. Thus, offspring that fledged in better body condition had mothers in better body condition, mothers with more colorful tails, and parents that fed them more often ($R^2 = 0.15$, $F^3_{134} = 7.5$, $P < 0.001$; Table 3).

To investigate which variables influenced the total number

TABLE 2. Comparisons of body condition and coloration of female eastern bluebirds of different ages. Data were standardized for year effects and values are means ± SD (sample sizes); comparisons are one-way MANOVAs.

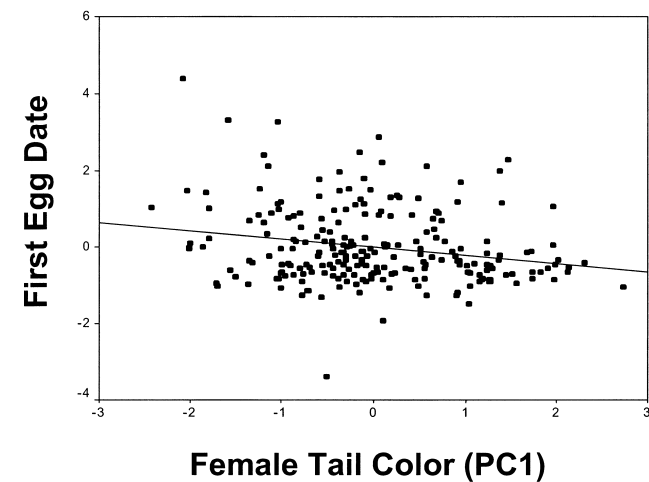| Character	1st year	2nd year	3rd year	4th year	5th year	F-statistic
Body condition	−0.07 ± 1.0 (93)	0.02 ± 1.0 (109)	0.24 ± 0.9 (21)	−0.19 ± 0.9 (10)	1.71 ± 2.4 (2)	$F^4_{230} = 0.66$
Breast color	−0.05 ± 1.1 (93)	0.01 ± 1.0 (109)	0.08 ± 1.0 (21)	0.34 ± 0.8 (10)	−0.74 ± 2.3 (2)	$F^4_{230} = 0.59$
Rump color	0.05 ± 1.1 (93)	−0.02 ± 0.9 (109)	0.13 ± 0.9 (21)	−0.33 ± 0.9 (10)	−0.47 ± 2.6 (2)	$F^4_{230} = 0.34$
Tail color	0.08 ± 0.7 (93)	−0.05 ± 1.0 (101)	−0.004 ± 1.0 (21)	0.13 ± 0.7 (10)	−0.2 ± 1.2 (2)	$F^4_{230} = 2.0$

FIG. 5. Relationship between female eastern bluebird tail coloration (standardized for year effects) and first egg date (day of year, standardized for year effects).

TABLE 3. Characteristics contributing to attributes of mate quality and reproductive success in eastern bluebirds. In each backward stepwise multiple regression model, only predictor variables that contributed significantly ($P < 0.05$) in the overall models are shown.

Attribute	Predictor variable	β
First egg date	Female tail color	−3.0
	Female age	−1.9
	Female body condition	−1.7
Maternal provisioning rate	Female rump color	2.2
	Female age	−2.1
	Female body condition	1.8
	Male rump color	1.7
Paternal provisioning rate	Female age	−1.9
Offspring condition	Female body condition	3.3
	Parental feeding rate	2.9
	Female tail color	1.6
Number offspring fledged	First egg date	−3.2
	Female age	2.6
	Female body condition	−2.0

of offspring that fledged from the nests of female eastern bluebirds, we constructed a backward stepwise multiple regression model. In this analysis, we included both male and female breast, rump, and tail color scores, body condition, maternal age, and first egg date as potential predictor variables. We found that first egg date, female age, and female body condition were significant predictors of the number of chicks fledged in a season. Thus, females that initiated egg laying earlier in the season (Fig. 6), older females, and females in poorer body condition fledged more offspring ($r^2 = 0.09$, $F^3_{196} = 6.7$, $P < 0.001$; Table 3). Measures of female and male plumage coloration were not significant predictors of number of offspring fledged.

DISCUSSION

Our study indicates that structural plumage coloration is a sexually selected signal of female quality in eastern bluebirds. The aviary data suggest that structural color in females has the potential to act as a reliable indicator of individual condition, and our field data demonstrate that coloration predicts female mate quality. The positive relationships between structural plumage ornamentation of females, maternal feeding rates, and offspring condition indicate that males could use expression of this trait to identify high quality partners, whereas trends between structural plumage ornamentation and first egg date suggest that females may use ornamentation to mediate competitive interactions over nest sites.

Our aviary experiment demonstrates that structurally based blue coloration in female bluebirds is sensitive to nutrition during molt. When we subjected female bluebirds to food deprivation, expression of structurally based coloration was significantly negatively impacted whereas expression of melanin coloration was not affected. These results indicate that structural and melanin coloration in female eastern bluebirds respond differently to restricted food access. Although the results are based on small sample sizes, we would have needed 60 birds in each experimental group for the relationship between melanin coloration and nutritional stress to have been significant given our effect size. Moreover, the obser-

vation that melanin coloration is insensitive to food deprivation is consistent with observations from other experimental studies with male songbirds (Gonzalez et al. 1999; Hill 2000; McGraw et al. 2002). Our observations indicate that even modest food restriction can affect structural plumage coloration—females in experimental and control groups differed in structural coloration but did not differ greatly in their weight loss before and after the experiment. These findings are consistent with male brown-headed cowbirds (*Molothrus ater*) in which structural, but not melanin-based plumage, was influenced by food deprivation (McGraw et al. 2002). Our observations are also consistent with field correlational studies of other species of passerine birds that have demonstrated relationships between measures of structural coloration and nutritional condition (Keyser and Hill 1999; Doucet 2002; Johnsen et al. 2003).

Our study is the first experimental test of the condition dependence of structural plumage coloration in females. In fact, although field correlations have shown associations be-
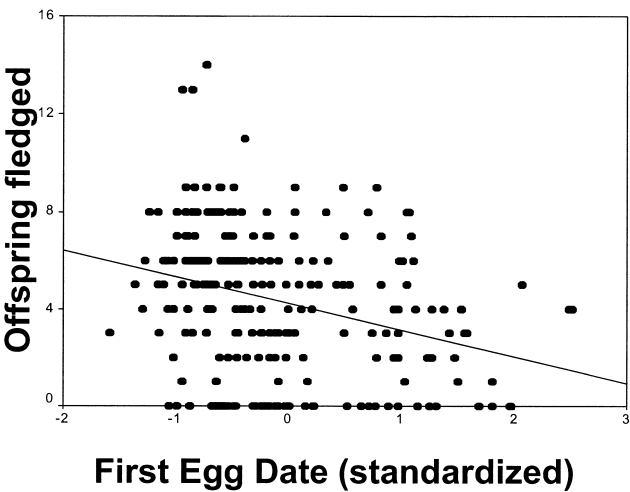

FIG. 6. Relationship between first egg date (day of year, standardized for year effects) and total number of offspring fledged in eastern bluebirds.

tween female ornamentation and parasitism (Potti and Merino 1996; Roulin et al. 2001) and between female ornamentation and body condition (Johnsen et al. 1996; Amundsen et al. 1997), only one other study has experimentally tested the condition dependence of ornamental traits in females (Hill 2002). That study used the same food deprivation design used in this study and showed a reduction in expression of carotenoid-based plumage coloration in female house finches subjected to nutritional stress.

Noniridescent blue structural coloration in birds is produced as a function of the size and arrangement of nanostructural elements within the medullary layer of feather barbs (Dyck 1971a,b; Prum et al. 1998, 2003). Eastern bluebird feathers are composed of a spongy medullary layer of feather barbs lying beneath a keratin cortex and above a layer of melanin granules surrounding large, central vacuoles (Shawkey et al. 2003). Transmission electron microscopy of feathers shows that males and females differ in many aspects of their feather structure, and birds expressing brighter color and greater UV chroma have thicker spongy layers and shorter distances between the scattering elements (i.e., keratin rods and air spaces; Shawkey et al. 2005). Perhaps only females with access to excellent nutrition during molt can produce feathers with more densely packed scattering elements and thicker spongy layers.

Evidence from the field suggests that structural coloration in female eastern bluebirds is related to female mate quality and reproductive success. Structural plumage coloration in females, characterized by greater UV chroma, more UV-shifted hues, and brighter coloration, is correlated with first egg date, maternal feeding rates, and offspring condition. Females with the more ornamented plumage coloration initiate egg laying earlier in the season, feed offspring more often, and fledge heavier young. The relationship between first egg date and female coloration indicates that coloration is likely to be a predictor of good physiological condition early in the season and suggests that coloration has an influence on either female-female competitive ability for access to breeding territories or male mate preference. Moreover, because first egg date was a strong predictor of annual reproductive success, the relationship between female plumage color and breeding date suggests an indirect link between plumage coloration and reproductive success in female eastern bluebirds.

In species exhibiting biparental care, measures of reproductive success are influenced by both female and male parental quality and the ornamentation of the mate may influence the investment strategies of each partner (Burley 1986). Thus, when we tested the relationship between female coloration and provisioning rates and measures of reproductive success, we included measures of male plumage coloration as possible predictors in the regression models. We found that females that exhibit more ornamented structural coloration feed offspring more often and also that females mated to males that exhibit more ornamented structural coloration feed offspring more often. The relationship between structural coloration in bluebirds and provisioning rates to offspring suggests plumage coloration is a reliable indicator of the quality of resources that both females and males can provide. Also this suggests that females mated to more or-

namented males may be more willing to allocate resources to offspring than females mated to duller males.

We found that female, rather than male color predicts offspring condition. Nestling mass at fledging is known to influence subsequent survival in passerines, with heavier offspring surviving better (Perrins 1965). Because more ornamented females fledge heavier offspring, female ornamentation may also be positively related to offspring survival. Similar relationships between female ornamentation and measures of female quality have been observed in a variety of taxa including breeding initiation date in birds (Ruusila et al. 2001), higher rates of maternal care in birds (Linville et al. 1998), and greater reproductive success in birds, fishes, and insects (Møller 1993; Berglund et al. 1997; Amundsen 2000; Bond[uriansky](...) 2001; LeBas et al. 2003; Massaro et al. 2003). Although some studies have failed to demonstrate relationships between female ornamentation and either greater reproductive success or parental effort (Hill 1993; Cuervo et al. 1995; Rohde et al. 1999; Smiseth and Amundsen 2000), large sample sizes may be necessary to detect weaker relationships.

Our field data also demonstrate that bluebirds tended to pair assortatively by tail coloration. Assortative mating by plumage coloration suggests that the most attractive male bluebirds mate with the most attractive female bluebirds. It is indirect evidence for sexual selection, but it supports the idea that female coloration could function via male mate choice or female-female competition for nest sites.

Although we found that experimental manipulations of food during molt affect structural plumage coloration in aviary experiments, we found no consistent relationships between body condition and plumage coloration in the field. In the field, we measured body condition in the spring, at the beginning of the breeding season rather than in the fall, during molt, so it is perhaps not surprising that body condition was unrelated to feather coloration created several months earlier. Females that display more-ornamented tails also have more-ornamented rumps, yet our field data indicate that tail and rump coloration are related to different measures of female quality. Although, the collinearity of rump and tail coloration probably creates this statistical artifact; our data suggest that tail coloration may be a better indicator of female quality than rump coloration.

The chestnut reflectance of breast feathers is created by combination of eumelanin and phaeomelanin pigments deposited in feathers. Male eastern bluebirds have higher overall melanin levels and higher relative amounts of eumelanin to phaeomelanin than do females (McGraw et al. 2004). Separate biochemical pathways create eumelanin and phaeomelanin color (Land and Riley 2000) and the two pathways are differentially sensitive to levels of circulating sex steroids (Hasse et al. 1995). Thus, sex steroid levels may explain variation in breast color among and between the sexes. Although we did not detect any significant relationships between melanin ornamentation and female quality, this does not definitively show that melanin plumage is not sexually selected. For example, females with darker and redder breast coloration may be signaling good genes for parasite resistance and immunocompetence as in the barn owl (*Tyto alba*; Roulin et al. 2000, 2001). Alternatively, melanin ornamen-

tation may act as a reliable signal of female competitive ability, as it is does in males of many species (reviewed by Senar 1999). In this study, we sampled birds after they had acquired nest sites. If female coloration functions primarily in acquisition of a breeding site, we might not have adequately sampled birds that lost in competition for nest sites. We also found negative covariance between melanin and structural ornamental traits in females, suggesting that birds may experience trade-offs between investing energy towards structural and melanin ornamentation. Male eastern bluebirds exhibit a similar negative relationship between melanin- and structurally based plumage ornamentation (Siefferman et al. 2005). Multiple sexually selected ornaments have traditionally been expected to be positively correlated with each other (Kodric-Brown and Brown 1984), but if allocation of energy is necessary for the production of both types of plumage coloration, negative correlations could arise (Andersson et al. 2002).

Age may also be an important criterion for male mate choice or may influence female-female competition for nest sites. We found strong assortative mating by age, and age was an important predictor of first egg date and reproductive success. In some species, age indicates greater parental ability, and females of many species increase in ornamentation as they age (Savalli 1995). Indeed, in male eastern bluebirds from the same population, structurally based plumage coloration and body condition increase with age (Siefferman et al. 2005). However, in females our data indicate that plumage color does not differ between the age classes.

An alternative hypothesis to explain ornamental plumage coloration in females is the correlated trait hypothesis, which proposes that female ornaments are co-influenced by strong selection for the traits in males, and hence that such female ornaments are selectively neutral or even detrimental (Lande 1980, 1987). However, both the nutritional stress experiment and the field data suggest that structural coloration in female eastern bluebirds serves a signaling function. Another alternative hypothesis was first proposed by Wallace (1889): females are vulnerable to predation while incubating eggs and thus are under stronger natural selection than males to be cryptic. Although nest predation is likely to constrain female coloration in many species (Martin and Badyaev 1996), selection for cryptic coloration in female bluebirds may be weak because they nest in cavities.

In conclusion, we found support for the hypothesis that structural plumage coloration of female eastern bluebirds is a reliable indicator of female quality. Female plumage coloration is correlated with first egg date, maternal care, and chick quality, so males could use expression of these traits to identify high quality partners. Moreover, we found a link between structural coloration in female bluebirds and nutrition during molt. Experimental tests could assess the importance of female plumage ornamentation on male choice and female-female competitive interactions in eastern bluebirds.

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