

A field test of female mate preference for male plumage coloration in eastern bluebirds

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A growing body of evidence shows that female birds use male plumage coloration as an important criterion in mate choice. In the field, however, males with brighter coloration may both compete better for high-quality territories and be the object of female choice. Positive associations between territory quality, male–male competitive ability and female preferences can make it difficult to determine whether females actively choose the most ornamented males. Male eastern bluebirds, *Sialia sialis*, display brilliant ultraviolet (UV)-blue plumage coloration on their heads, backs, wings and tails and chestnut coloration on their breasts, which is positively correlated with condition, reproductive effort and reproductive success. We tested the hypothesis that female bluebirds prefer males that display brighter and more chromatic coloration by experimentally widowing males in the field and allowing replacement females to choose partners. We controlled for the influence of territory quality on female choice by widowing dyads of males with adjacent territories. We found no evidence that UV-blue or chestnut plumage coloration, body size or body condition predicted the male with which a female would pair. We found no support for the hypothesis that the coloration of male eastern bluebirds functions as a criterion in female mate choice.

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It is generally thought that the bright plumage coloration displayed by males of many bird species evolved in response to selection from female choice for elaborate displays (Hill 2006). Indicator models of sexual selection propose that the expression of sexually selected traits, such as plumage coloration, reliably signal individual condition (Andersson 1994; Hill 2002). These traits can act as honest signals of an individual's phenotypic or genetic quality if only exceptionally fit individuals in a population achieve maximum expression of such traits (Zahavi 1975; Hamilton & Zuk 1982; Kodric-Brown & Brown 1984). Indicator models predict that individuals that display more exaggerated traits compete better for mates and thus experience higher reproductive success. There is now substantial experimental and correlational evidence that, in some species of birds, females

discriminate between potential mates by assessing colourful plumages (reviewed in Hill 2006).

Studies of female mate choice in birds range from weak correlative field studies and uncontrolled aviary observations to carefully controlled experimental tests in both the field and the laboratory. Laboratory experiments can be useful because they allow researchers to manipulate coloration and disassociate colour traits from other correlated traits. Eliminating the confounding influence of male–male competition, for example, can be very helpful (Wagner 1998). However, eliminating male–male competitive interactions usually necessitates that males are housed individually and thus reduces physical access to females. When females do not have physical access to potential mates and cannot copulate with males, researchers generally use association time as a proxy for mate choice. Association time, however, may not always be an accurate and consistent measure of mate preference (Hill 2006). Females also may not make mate-choice decisions in the relatively unnatural environment of the laboratory. Finally, in laboratory-based mate-choice trials, it becomes difficult for researchers to investigate the fitness consequences of mating decisions (Hill 2006). For example, for most species in the laboratory, it is not possible to examine how the chosen male contributes to the female's breeding success. Contributions to reproductive success

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include direct benefits (territory quality and parental care) and indirect benefits (fertilization rate and genetic quality) provided by males. Controlled tests of female preferences conducted in the field provide an alternative to laboratory-based trials and can allow researchers to quantify both female choice and the ramifications of those choices.

The eastern bluebird, *Sialia sialis*, is among the better studied bird species with respect to the function of ornamental plumage coloration, and yet the importance of male coloration as a criterion in female mate choice remains uncertain. Male bluebirds display a structural UV-blue coloration on the plumage of their backs, heads, wings and tails and chestnut melanin coloration on their breasts compared with the overall duller coloration in females. Although no studies have examined visual perception in eastern bluebirds, UV vision has been shown in closely related species (Hart et al. 2000) and UV-blue coloration in passerine birds has been suggested to play an important role in signal communication (Hunt et al. 1999; Hill 2006). A correlative field study of eastern bluebirds shows that male eastern bluebirds with brighter UV-blue plumage and darker melanin breast colour pair earlier in the season, feed chicks more often, and gain higher reproductive success (Siefferman & Hill 2003). Experimental manipulations of paternal investment show that UV-blue structural coloration is a condition-dependent trait in bluebirds (Siefferman & Hill 2005a). Although the field correlative study linking coloration, earlier pairing and reproductive success suggests that females may use coloration to choose mates, experimental manipulations of structural coloration in an aviary show no consistent relationship between male UV-blue coloration and female association time (Liu et al. 2007).

Bluebirds are obligate cavity-nesting passerines and males that express more-ornamented UV-blue coloration are more successful competitors for limited nest sites (Siefferman & Hill 2005b). Thus, in the eastern bluebird as in many species that defend key resources necessary for reproduction, female choice for male coloration is confounded by female choice for the superior resources defended by brighter males. Simple correlative studies cannot distinguish the relative influences of female choice of mate and male–male competition in driving the elaboration of plumage coloration.

To test female preferences for ornamental plumage coloration more directly in a wild population of breeding eastern bluebirds, we conducted a mate-removal experiment. We removed the original mates from males to quantify the latency time for males to establish new pair bonds. We used this protocol to avoid any bias associated with prior pair bonds, because past relationships can confound mate choice in two ways. Pairs that have previous breeding experience in the same territory might experience advantages in acquiring nesting sites (Qvarnstrom & Forsgren 1998) and in reproductive success (Ligon 1999). We removed the mates of two males that held neighbouring territories with the assumption that males with adjacent territories held territories of similar quality. This experiment was designed to measure female preference in the wild and to quantify the breeding success of the subsequent mate selection on a platform of equal-quality territories.

First, by removing the original mates early in the breeding season, we caused pairs to reconstruct the early breeding season processes of mate choice and pairing. Territories from which females had been removed represented available males. Second, we investigated the characteristics of the males and determined which traits predicted male attractiveness to females. We tested the hypothesis that female eastern bluebirds prefer to pair with males that display the more-ornamented plumage coloration. Additionally, by monitoring the behaviour of the newly formed pairs throughout the breeding season, we quantified reproductive success.

METHODS

General Methods

Breeding cavities are probably the key resource that limits reproduction in eastern bluebirds (Gowaty & Plissner 1998). Nonbreeding birds (floaters) are present in suitable habitats, are sexually mature and display breeding condition (M. Liu, personal observation). When breeding opportunities become available (the original box owners die or disappear from a territory), these floaters frequently assume the territory and breed with widowed birds.

This study was conducted from March to August of 2005 and 2006 in two field sites, located in southeastern Alabama, U.S.A., with a total of 200 nestboxes. The distance between nestboxes was approximately 100 m. The habitat consisted mainly of pastures and hay fields surrounded by fragmented pine forests.

In this long-term research population, the birds were captured and banded in the early breeding season (1–15 March), before the experiment began. At each field site, we also monitored all other unmanipulated territories. To capture birds, we lured pairs into mist nets by playing recordings of eastern bluebird calls. Each bird was individually marked with a unique combination of three coloured bands and one U.S. Fish and Wildlife Service aluminium band. We measured body mass (accuracy 0.1 g) and the length of tarsi, wings and tails (accuracy 0.5 mm). We used principle component (PC) analysis to reduce our morphological measurements (tarsus, wing, tail) into PC scores. Principal component 1 explained 62.5% of the variation in morphological measurements. Body condition was calculated as the residuals of a regression of body mass on PC1 of body size (Jakob et al. 1996).

At the time of capture, we carefully plucked 8–10 rump (blue) and breast (chestnut) feathers. Feathers were placed in envelopes and stored in a temperature- and humidity-controlled laboratory before analysis. We taped feathers onto black paper (Canson cat. no. 425 Stygian black) using an overlapping fashion to mimic how feathers naturally occur on the birds. We measured the plumage reflectance using an Ocean Optics S2000 spectrometer and deuterium–tungsten halogen light source (range 250–880 nm). We used a fibre-optic cord equipped with a black rubber cap on the metal probe to exclude ambient light. The distance between the probe and the feather was set to 5 mm to create a measurement diameter of approximately 2 mm. The probe was perpendicular to the feather surface. We took all colour measurements at the same setting and the same person (M. L.) processed all data. We generated reflectance data relative to a white standard (Labsphere, Inc.) using OOIBase software (Ocean Optics). We measured each feather sample five times and used the mean to generate colour scores for each male.

For chestnut coloration (Fig. 1), we summarized reflectance data by calculating two standard descriptors of reflectance spectra: brightness and chroma. Mean brightness was calculated as the mean summed reflectance ($R_{300-700\text{nm}}$). Red chroma was calculated as the proportion of the total reflectance ($R_{300-700\text{nm}}$) in the red part of the spectrum ($R_{575-700\text{nm}}$). For UV-blue colour (Fig. 1), we calculated three standard descriptors of reflectance spectra: brightness, chroma and hue. Mean brightness was calculated as $R_{300-700\text{nm}}$. UV chroma was calculated as the proportion of $R_{300-700\text{nm}}$ in the UV part of the spectrum ($R_{300-400\text{nm}}$). Blue chroma was calculated as the proportion of $R_{300-700\text{nm}}$ in the blue part of the spectrum ($R_{400-512\text{nm}}$). Hue was calculated as the wavelength at peak reflectance. Calculations taken from the same spectral curves are correlated in eastern bluebirds such that the most ornamented UV-blue males display brighter coloration, greater UV chroma, and hues with wavelengths shifted towards the shorter wavelengths

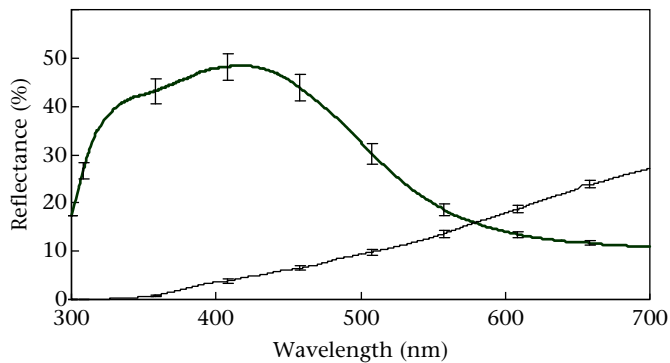


Figure 1. Mean (± 1 SE) reflectance spectra of the UV-blue of rumps (thick line) and the chestnut coloration of breasts (thin line) of male eastern bluebirds ($N = 16$).

(Siefferman et al. 2005). Moreover, the most ornamented chestnut males display darker coloration (lower brightness) and greater red chroma.

Female Removal

In both years, we conducted the experiment within 3 weeks of the first egg date in the population (1–15 March 2005 and 15 March–1 April 2006). For each removal, we chose two adjacent territories (within 200 m of each other) such that each territory had a resident pair and the two pairs were synchronized in their breeding cycles (the females began laying eggs on the same date ± 2 days). Within 5 days of the date on which the females of two adjacent pairs began laying eggs, we captured the males and females of both pairs. On the morning of the capture, we released the males on their original territory within 30 min of capturing them and placed each female in captivity. Upon capture, the eggs and nest materials were collected from the nestboxes. In each year, the manipulated dyads of territories were geographically scattered at two study sites and occurred near or interspersed with other eastern bluebird territories where breeding pairs remained unmanipulated. Each day, we removed no more than two dyads of females.

We monitored the behaviour of the experimentally widowed males twice daily and determined the date in which they attracted a new female to their territory. After each male had secured a new mate, we monitored the breeding activities of the newly formed pair throughout the breeding season. To reduce disturbance, replacement females were captured during the late incubation or early nestling stages.

Behavioural Observations

We began behavioural observations of focal males within 12 h of the time of female removal. Each day after the removal, we monitored the behaviour of each focal male in the morning (60 min) to determine whether the male had attracted a replacement mate. During morning observations, we recorded male song rate (defined as number of songs per minute) for approximately 30 min. The open grassland habitat and propensity of bluebirds to perch in the open, near their nestboxes, allowed for detection of new females. We determined that a new female had arrived if the female was seen with the male for a minimum of 30 continuous minutes and if the female was seen with the focal male for 2 consecutive days. We digitally photographed pairs to confirm the identity of the newly arrived female. After one of the males from each dyad had successfully attracted a new female, we reduced our observation frequency to once every 2 or 3 days and continued to monitor all

the territories until the end of the breeding season. For all subsequent nests, we recorded the date of the first laid egg of each brood, the clutch size, the brood size and the fledging success.

After a female was removed from the original territory, widowed males sang and exhibited display behaviours on or near the nestboxes (perched on boxes, repeatedly looked into nest holes, waved wings and carried nesting material). We recorded the song rates of males during morning observations in 2006. Because some males attracted females quickly, and because song rate and display behaviours were altered by the presence of the new females, we were able to record singing behaviour of only 10 males. Song rate did not correlate with plumage colour (all $P > 0.1$), so we eliminated song as a variable in subsequent analyses.

When newly arrived females were visible, we recorded behaviours indicative of mating interest including when females foraged in the males' territory, perched on boxes, repeatedly looked into nest holes, or followed (or was followed by) the male of the territory. Mate-guarding behaviours are unambiguous in this species (pairs are usually observed within 20 m of each other during courtship and egg laying) and we are confident that we could accurately determine when a male paired with a female (L.S., personal observation). We used two measurements to describe the males' success in attracting new females: the latency period between the removal of the female and the attraction of a new mate (LA1; in days) and the time lapse between the date of the original female's removal and the date on which the replacement female commenced egg laying (LA2). We considered each dyad of widowed males to represent one trial. During the study, most of the males ($N = 40$) were used only once. However, four males were used in both 2005 and 2006, but all males were paired with new mates, occupied new territories, and were placed in dyads with unique males. In each trial, the male 'won' if he attracted a female to his territory before the other male in the dyad ('winning' males showed shorter LA1 than 'losing' males). In two cases, we missed the exact date that the winner attracted his female, but they were assigned by the first egg date of their mate.

Ethical Note

This study was conducted with approval from the Institutional Animal Care and Use Committee (PRN: 2003-0448) at Auburn University. After capture, females were housed in outdoor flight cages of a permanent aviary at Auburn University (for details see Liu et al. 2007). Birds were given water ad libitum and live mealworms and crickets. Our aviary setting effectively reduced the cage stress; we bred a pair of eastern bluebirds successfully in such an aviary cage in 2004. All birds were maintained in the aviary for approximately 4–6 weeks and then released to their original territories in the breeding season (mid-May) after the experiment. Because most bluebird pairs initiate second broods after mid-May, we expected that any females that could find a mate at this time had the opportunity to breed later in the season. We did observe that a released female bred successfully with a new male in June of 2006. Additionally, we recorded the return rate of the original (17.3%) versus the replacement females (21.7%) in 2007. Captivity did not influence the likelihood that females would return to breed at the field site in the following year (Fisher's exact test: $P = 0.73$).

Statistical Analyses

We used SPSS 11.5 (SPSS for Windows; SPSS, Inc., Chicago, IL, U.S.A., 2003) for all analyses. We used paired t tests to compare male characteristics within each dyad. Additionally, we combined all data and used backwards and forwards regression models to examine whether male characteristics predicted female arrival

time, time to breed and total seasonal reproductive success (number of offspring fledged). All data conformed to normality (Shapiro–Wilk's tests), and parametric tests were used. Blue brightness differed by year, thus we standardized these data for year ($X \pm SD = 0 \pm 1$). All significance levels were two-tailed. Sample sizes vary between some variables because we failed to collect feathers and morphological measurements for three males, and we did not collect plumage data from 10 females.

RESULTS

Paired Comparisons

Over the course of two breeding seasons, we conducted 24 trials of female removals (females were removed from 48 territories). Forty-one widowed males attracted a new mate after mate removal and all of these newly formed pairs bred successfully later in the breeding season. Four males disappeared from their territories after their original mates were removed. One female escaped from the aviary and reunited with her original mate. Of 19 successful trials, two yielded 'tie' results (both males attracted replacement mates on the same day) and were eliminated from the paired comparisons. The median LA1 was 3 days (range 0.5–58 days), and the median LA2 was 13 days (range 7–63 days). LA1 and LA2 did not correlate ($r_s = 0.33$, $N = 31$, $P = 0.07$).

Paired t tests revealed that within dyads, there were no significant differences between the UV-blue coloration of the rumps of winning and losing males (winners–losers; UV chroma: mean difference = -0.01 ± 0.03 , $t = 1.16$, $N = 15$, $P = 0.27$; blue chroma: mean difference = -0.001 ± 0.02 , $t = 0.20$, $N = 15$, $P = 0.85$; brightness (z): mean difference = 0.34 ± 1.34 , $t = 0.98$, $N = 15$, $P = 0.34$; hue: mean difference = 3.94 ± 19.76 , $t = 0.77$, $N = 15$, $P = 0.45$; Table 1). Nor did we find a significant difference in chestnut coloration of the breast of winning and losing males (red chroma: mean difference = -0.01 , $t = 1.04$, $N = 13$, $P = 0.32$; brightness: mean difference = 0.38 , $t = 0.67$, $N = 13$, $P = 0.52$; Table 1). Further, morphological measurements did not differ significantly between winning and losing males (body size: mean difference = 0.19 ± 1.23 , $t = 0.56$, $N = 13$, $P = 0.59$; body condition: mean difference = 10.64 ± 135.6 , $t = 0.28$, $N = 13$, $P = 0.78$; Table 1). Over the course of the breeding seasons, the winning and losing males fledged a similar number of chicks (mean difference = 1.08 ± 3.90 , $t = 0.96$, $N = 12$, $P = 0.36$; Table 1).

Because the manipulations that we conducted were technically difficult, our sample sizes were relatively small. Thus we used power analyses to determine whether our sample sizes were sufficient to find an effect if it existed. Previous studies that were laboratory based found that $\geq 80\%$ of females preferred the males with the more-ornamented structurally based plumage (Bennett et al. 1997; Hunt et al. 1999; Siitari et al. 2002). Given our sample sizes ($N = 17$), we calculated that, if female bluebirds showed a preference similar to that of female blue tits and starlings, we had an 82% likelihood of finding an effect with our data set.

Male Comparisons

We treated each territory as an independent event and investigated the potential relationships between male characteristics, measures of mate attraction and breeding success. We found no significant effects of year on either LA1 (t test: $t = 1.51$, $N = 15$, 22 , $P > 0.1$) or LA2 ($t = 1.20$, $N = 15$, 24 , $P > 0.1$).

All territories were manipulated in a relatively short period (3 weeks), and we found no influence of removal date on LA1 (2005: $r_s = 0.37$, $N = 15$, $P = 0.17$; 2006: $r_s = 0.16$, $N = 22$, $P = 0.49$) nor on LA2 (2005: $r_s = -0.10$, $N = 15$, $P = 0.72$; 2006: $r_s = 0.1$,

Table 1

Comparisons of characteristics of all males that gained a mate first (won) or second (lost)

Trait	Outcome	Mean	SD	N	t	P
Tarsus (mm)	Won	20.48	0.65	16	-0.02	0.99
	Lost	20.48	0.73	16		
Wing (mm)	Won	101	2.27	16	0.25	0.81
	Lost	100.78	2.89	16		
Tail (mm)	Won	64.47	2.82	16	1.83	0.09
	Lost	62.91	2.58	16		
Mass (g)	Won	29.61	1.11	15	0.07	0.94
	Lost	29.55	2.75	15		
Body condition (z)	Won	1.45	0.07	15	0.28	0.78
	Lost	1.45	0.11	15		
Age (years)	Won	1.83	0.39	12	0.8	0.44
	Lost	1.67	0.49	12		
Rump brightness (z)	Won	0.24	1.01	15	0.98	0.34
	Lost	-0.10	0.94	15		
Rump UV chroma (z)	Won	0.34	0.02	15	-1.16	0.27
	Lost	0.35	0.02	15		
Rump blue chroma (z)	Won	0.40	0.01	15	-0.2	0.85
	Lost	0.40	0.01	15		
Rump hue (z)	Won	417.88	12.37	15	0.77	0.45
	Lost	413.93	9.12	15		
Breast brightness (z)	Won	10.12	1.20	13	0.67	0.52
	Lost	9.74	1.68	13		
Breast red chroma (z)	Won	0.63	0.03	13	-1.03	0.32
	Lost	0.64	0.02	13		
LA1 (days)	Won	3.00	2.56	11	-3.23*	0.001
	Lost	11.36	15.98	11		
LA2 (days)	Won	12.77	3.32	13	-2.03*	0.04
	Lost	22.77	17.58	13		
Breeding success (young)	Won	6.83	2.08	12	0.96	0.36
	Lost	5.75	2.90	12		

Significant P values are shown in bold.

*Paired t test.

$N = 24$, $P = 0.65$). Additionally, because males of similar quality were more likely to occupy neighbouring territories, we calculated the average colour of males within dyads to test whether the average dyad coloration predicted average time to attract new females. We found no significant relationship in any comparison (Table 2). Finally, when we used a mixed-model analysis with dyad identification as a random factor, we found no significant relationship between male coloration and female arrival latency (UV-blue rump brightness: $F_{1,33} = 1.85$, $P = 0.18$; UV chroma: $F_{1,33} = 0.29$, $P = 0.60$; blue chroma: $F_{1,33} = 0.19$, $P = 0.67$; hue: $F_{1,33} = 0.19$, $P = 0.67$; chestnut breast brightness: $F_{1,24} = 2.91$, $P = 0.10$; red chroma: $F_{1,25} = 0.88$, $P = 0.36$).

We used backwards multiple regression models to determine the influence of male colour measurements and morphology on (1) LA1, (2) LA2 and (3) the total offspring number of fledged. We found no significant influence of male coloration, body size or body condition on latency to attract new females (model included UV-blue brightness, chestnut brightness, and body size: R^2 adj = 0.05, $F_{3,-21} = 1.41$, $P = 0.27$; Fig. 2), latency for new females to begin breeding (model included chestnut red chroma, body condition and body size: R^2 adj = 0.04, $F_{3,-24} = 1.41$, $P = 0.27$; Fig. 2) or total number of offspring fledged in that season (model included UV-blue hue, chestnut brightness, chestnut red chroma: R^2 adj = 0.07, $F_{3,-24} = 1.69$, $P = 0.20$; Fig. 3).

Table 2

Correlations between the mean plumage coloration of males in each dyad and the mean latency to attracting a mate for each dyad

Trait	r_s	N	P
Mean rump brightness (z)	0.44	16	0.09
Mean rump UV chroma (%)	-0.13	16	0.63
Mean rump blue chroma (%)	0.27	16	0.31
Mean breast brightness (%)	-0.34	15	0.22
Mean breast red chroma (%)	0.24	15	0.39

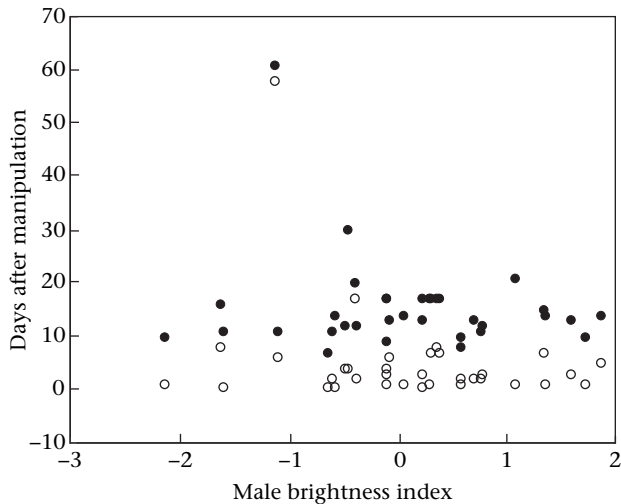


Figure 2. Relationship between brightness of UV-blue rump coloration of the male eastern bluebirds and time to acquire a new mate (open circles) and time to begin breeding with new mate (filled circles). Colour data are standardized for year.

To investigate these relationships further, we used a series of forward multiple regression models to explore the potential influences of male characteristics on LA1 and LA2. The first model included only the six measurements of male plumage coloration as predictor variables and we found that coloration did not influence LA1 (full model: R^2 adj = 0.12, $F_{5,26} = 0.71$, $P = 0.62$) or LA2 (full model: R^2 adj = 0.12, $F_{5,28} = 0.74$, $P = 0.6$). In the next model, we included only male body size and body condition and, again, found no influence of male morphological characteristics on LA1 (full model: R^2 adj = 0.10, $F_{2,25} = 1.42$, $P = 0.26$) or LA2 (full model: R^2 adj = 0.10, $F_{2,25} = 1.58$, $P = 0.22$). Finally, a large model that included all measures of colour and morphology found no influence on LA1 (full model: R^2 adj = -0.05, $F_{5,26} = 0.71$, $P = 0.62$) or LA2 (full model: R^2 adj = -0.04, $F_{5,28} = 0.74$, $P = 0.60$).

Finally, we also used discriminate function analyses (DFA) to identify any unique characteristics of winning and losing males. We combined all colour variables and morphological traits into these models. We found no significant difference between winning and losing group (Wilk's λ $F_{7,27} = 5.19$, $P = 0.64$). Based on previous

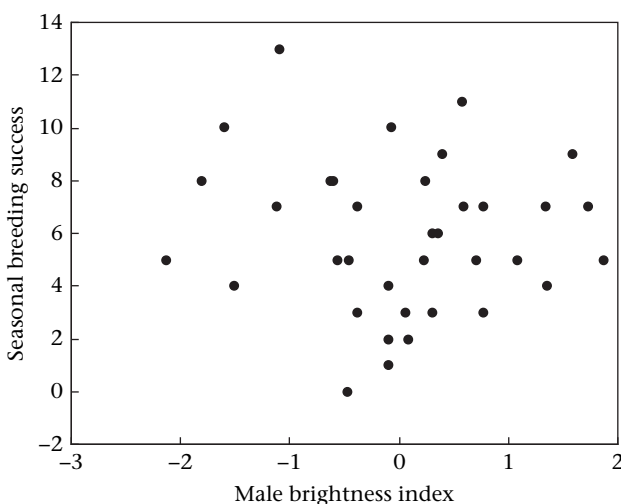


Figure 3. Relationship between brightness of UV-blue rump coloration of the male and seasonal reproductive success (total number of nestlings fledged per year) of eastern bluebirds. Colour data are standardized for year.

studies of the same population (Siefferman & Hill 2005b), we selected three predictors (UV-blue brightness, UV chroma and body size) for another DFA. Again, we found that the overall model was not significant (Wilk's λ $F_{3,28} = 3.54$, $P = 0.32$), indicating that there was no significant difference in morphology or coloration of winning and losing males.

Assortative Mating

Because pairs may mate assortatively for coloration, we tested whether the mated pairs' plumage and body size characteristics were more similar than random using Pearson's correlations. We found no significant relationships between the UV-blue colour (brightness $r = 0.02$, $N = 35$, $P = 0.91$; UV chroma $r = -0.03$, $N = 35$, $P = 0.85$; blue chroma $r = 0.02$, $N = 35$, $P = 0.93$; hue $r = 0.13$, $N = 35$, $P = 0.47$), the chestnut colour (brightness $r = 0.02$, $N = 32$, $P = 0.93$; red chroma $r = 0.03$, $N = 32$, $P = 0.89$) or the body size ($r = 0.06$, $N = 38$, $P = 0.72$) of the original female and her mate. Nor did we find any significant relationships between the UV-blue (UV-blue rump: brightness $r = -0.13$, $N = 25$, $P = 0.55$; UV chroma $r = 0.09$, $N = 25$, $P = 0.67$; blue chroma $r = -0.16$, $N = 25$, $P = 0.43$; hue $r = 0.01$, $N = 25$, $P = 0.95$), the chestnut colour (brightness $r = 0.29$, $N = 24$, $P = 0.17$; red chroma $r = 0.02$, $N = 24$, $P = 0.93$) or the body size ($r = 0.06$, $N = 25$, $P = 0.78$) of the replacement female and her mate.

DISCUSSION

Contrary to our hypothesis that female bluebirds prefer to pair with males that display the most ornamented structural coloration, we found no evidence of mate choice based on plumage coloration. When we removed females and created experimentally widowed males, unmated females quickly settled in the opened territories. We found no evidence that females preferentially settled with the more-ornamented males. In comparisons of dyads of males that were carefully matched for territory quality and for timing of mate removal, the more-ornamented and larger males did not attract females more quickly compared to the less-ornamented and smaller males. Although we did not find evidence for a female preference, it is possible that a much larger sample size might have shown a trend.

The observation from our field manipulation that female eastern bluebirds do not use male coloration as a criterion in mate choice is consistent with aviary-based mate choice trials in which female eastern bluebirds chose males without regard to either manipulated UV-blue plumage coloration or natural variation in UV-blue plumage coloration (Liu et al. 2007). The lack of influence of male body size, body condition, or chestnut coloration on female mate-choice decisions is also consistent with the results of the aviary-based trials (Liu et al. 2007; M. Liu, L. Siefferman & G. Hill, unpublished data).

Our experimental design differs from most approaches to mate choice in three important ways. First, unlike most field-based tests of mate choice, we matched pairs of widowed males on adjacent territories. This design was used to control for territory quality, as we assume that, in the relatively homogeneous grassland habitat of eastern bluebirds, males with adjacent territories will have territories of similar quality. Second, unlike most field-based research on mate choice, we removed the original females, thus eliminating any influence of the pair's prior breeding experience. Third, unlike most laboratory-based experiments of mate choice, our design is unique because females were free to interact with males, and to reproduce, and we quantified subsequent reproductive success.

Past research with bluebirds shows that males that display more exaggerated blue and chestnut coloration pair earlier and feed

offspring more often (Siefferman & Hill 2003), suggesting that female choice of more elaborately ornamented males would be adaptive. Further, Siefferman & Hill (2005b) found that UV-blue structural coloration is a good indicator of the ability of males to secure limited nestboxes, suggesting that males with more elaborate UV-blue coloration have advantages in competitive interactions for breeding sites. In this population, however, older male birds also tend to display brighter UV-blue coloration and duller chestnut coloration compared to younger males (Siefferman et al. 2005). The positive correlations between plumage coloration and reproductive success in eastern bluebirds could be a consequence of older pairs experiencing greater reproductive success because of increased mate familiarity and reproductive experience (Black 1996). Indeed, in this population, pairs mate assortatively by age and older females tend to breed earlier and experience greater seasonal reproductive success (Siefferman & Hill 2005c).

The newly formed pairings that resulted from our experiment might have disentangled the past interrelationships between length of pair bond, age of birds, male plumage coloration and reproductive success (Siefferman & Hill 2003). Perhaps females prefer to nest with males with which they have had prior nesting or social experience and those males tend to express greater UV-blue coloration because they tend to be older. The lack of effect of colour on timing of attraction of replacement mates suggests that females are not actively seeking more colourful males.

Several studies have found that pair duration and previous breeding experience are important determinants of reproductive success in socially monogamous species (Ligon 1999) and past pair bonds are probably important in this population of bluebirds. The majority of the males that were experimentally widowed successfully attracted females. In addition to experimentally manipulated pairs, we banded and monitored the nesting attempts of all bluebirds that bred on our study site (80 pairs) and found that no females left their original mates to pair with experimentally widowed males. Moreover, all replacement females arrived on our field site without bands, suggesting that those females came from the floating pool of birds.

Our data are consistent with research suggesting that the cost of leaving a mate is generally greater than the benefits gained from pairing with a higher-quality mate (Ligon 1999; but see Otter & Ratcliffe 1996). In general, females that leave their mates experience the costs of delayed breeding (Black et al. 1996). Because species that nest exclusively in secondary cavities have historically experienced very limited nest sites, females that choose to leave their original mates face the risk of forsaking all breeding opportunities in that breeding season (Gowaty 1981). In our population, most pairs breed from March to August and are able to produce two or three successful broods. Pairs that initiate egg laying earlier in the season experience greater seasonal reproductive success (Siefferman & Hill 2005c). Indeed, in swans, mate familiarity has been shown to reduce the costs of courtship and facilitate earlier breeding (Rees et al. 1996).

Our experimental design helped us disentangle the confounding effects of coloration, prior pairing history, and reproductive success that may naturally occur in eastern bluebirds. We found no evidence that female eastern bluebirds seek out more colourful males as mates. We also found no evidence that pairs mate assortatively for coloration or body size; again, this is consistent with field correlational evidence (Siefferman & Hill 2005c). It is possible, however, that female bluebirds may not all display similar mate-choice preferences. Several researchers have shown that female mating preference might vary depending on environment, prior experience, and body condition (reviewed in Qvarnstrom 2001). It is also possible that coloration functions in male–male interactions and signals male condition. Further, it is possible that females use

male plumage coloration to choose extrapair mates, as has been found in the sister species, mountain bluebirds, *Sialia currucoides* (Balenger et al. 2009). Future research should investigate individual variation in mate preferences and the signalling function of male coloration and genetic quality in eastern bluebirds (Mays & Hill 2004; Mays et al. 2008).

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References

- Andersson, M. 1994. *Sexual Selection*. Princeton, New Jersey: Princeton University Press.
- Balenger, S. L., Johnson, L. S. & Masters, B. S. 2009. Sexual selection in a socially monogamous bird: male color predicts paternity success in the mountain bluebirds *Sialia currucoides*. *Behavioral Ecology and Sociobiology*, **63**, 403–411.
- Bennett, A. T. D., Cuthill, I. C., Partridge, J. C. & Lunau, K. 1997. Ultraviolet plumage colors predict mate preferences in starlings. *Proceedings of the National Academy of Sciences, U.S.A.*, **94**, 8618–8621.
- Black, J. M. 1996. *Partnerships in Birds: the Study of Monogamy*. Oxford: Oxford University Press.
- Black, M. J., Choudhury, S. & Owen, M. 1996. Do barnacle geese benefit from lifelong monogamy? In: *Partnerships in Birds* (Ed. by J. M. Black), pp. 91–117. Oxford: Oxford University Press.
- Gowaty, P. A. 1981. An extension of the Orians–Willson model to account for mating systems besides polygyny. *American Naturalist*, **118**, 851–859.
- Gowaty, P. A. & Plissner, J. H. 1998. Eastern bluebird. In: *Birds of North America* (Ed. by A. Poole & F. Gill). Philadelphia: The Birds of North America.
- Hamilton, W. D. & Zuk, M. 1982. Heritable true fitness and bright birds: a role for parasites? *Science*, **218**, 384–386.
- Hart, N. S., Partridge, J. C., Cuthill, I. C. & Bennett, A. T. D. 2000. Visual pigments, oil droplets, ocular media and cone receptor distribution in two species of passerine bird: the blue tit (*Parus caeruleus* L.) and the blackbird (*Turdus merula* L.). *Journal of Comparative Physiology A*, **186**, 375–387.
- Hill, G. E. 2002. *A Red Bird in a Brown Bag: the Function and Evolution of Ornamental Plumage Coloration in the House Finch*. New York: Oxford University Press.
- Hill, G. E. 2006. Female mate choice for ornamental coloration. In: *Bird Coloration. Vol. II: Function Evolution* (Ed. by G. E. Hill & K. McGraw), pp. 137–200. Cambridge, Massachusetts: Harvard University Press.
- Hunt, S., Cuthill, I. C., Bennett, A. T. D. & Griffiths, R. 1999. Preferences for ultraviolet partners in the blue tit. *Animal Behaviour*, **58**, 809–815.
- Jakob, E. M., Marshall, S. D. & Uetz, G. W. 1996. Estimating fitness: a comparison of body condition indices. *Oikos*, **77**, 61–67.
- Kodric-Brown, A. & Brown, J. H. 1984. Truth in advertising: the kinds of traits favored by sexual selection. *American Naturalist*, **124**, 309–323.
- Ligon, J. D. 1999. *The Evolution of Avian Breeding Systems*. Oxford: Oxford University Press.
- Liu, M., Siefferman, L. & Hill, G. E. 2007. An experimental test of female choice relative to male structural coloration in eastern bluebirds. *Behavioral Ecology and Sociobiology*, **61**, 623–630.
- Mays, H. L. & Hill, G. E. 2004. Choosing mates: good genes versus genes that are a good fit. *Trends in Ecology & Evolution*, **19**, 554–559.
- Mays, H. L., Albrecht, T., Liu, M. & Hill, G. E. 2008. Female choice for genetic complementarity in birds: a review. *Genetica*, **134**, 147–158.
- Otter, K. & Ratcliffe, L. 1996. Female initiated divorce in a monogamous songbird: abandoning mates for males of higher quality. *Proceedings of the Royal Society B*, **263**, 351–354.
- Qvarnstrom, A. 2001. Context-dependent genetic benefits from mate choice. *Trends in Ecology & Evolution*, **16**, 5–7.
- Qvarnstrom, A. & Forsgren, S. 1998. Should females prefer dominant males? *Trends in Ecology & Evolution*, **13**, 498–501.
- Rees, C. E., Liesvieser, P., Pettifor, A. R. & Perrins, C. 1996. Mate fidelity in swans: an interspecific comparison. In: *Partnerships in Birds* (Ed. by J. M. Black), pp. 118–137. Oxford: Oxford University Press.

- Siefferman, L. & Hill, G. E.** 2003. Structural and melanin coloration indicate parental effort and reproductive success in male eastern bluebirds. *Behavioral Ecology*, **14**, 855–861.
- Siefferman, L. & Hill, G. E.** 2005a. Male eastern bluebirds trade future ornamentation for current reproductive investment. *Biology Letters*, **1**, 208–211.
- Siefferman, L. & Hill, G. E.** 2005b. UV-blue structural coloration and competition for nestboxes in male eastern bluebirds. *Animal Behaviour*, **69**, 67–72.
- Siefferman, L. & Hill, G. E.** 2005c. Evidence for sexual selection on structural plumage coloration in female eastern bluebirds. *Evolution*, **59**, 1819–1828.
- Siefferman, L., Hill, G. E. & Dobson, F. S.** 2005. Ornamental plumage coloration and condition are dependent on age in eastern bluebirds *Sialia sialis*. *Journal of Avian Biology*, **36**, 428–435.
- Siitari, H., Honkavaara, J., Huhta, E. & Viitala, J.** 2002. Ultraviolet reflection and female mate choice in the pied flycatcher, *Ficedula hypoleuca*. *Animal Behaviour*, **63**, 97–102.
- Wagner, W. E.** 1998. Measuring female mating preferences. *Animal Behaviour*, **55**, 1029–1042.
- Zahavi, A.** 1975. Mate selection—a selection for a handicap. *Journal of Theoretical Biology*, **53**, 205–214.