THE RELATIONSHIP BETWEEN CAROTENOID-BASED COLORATION AND PAIRING, WITHIN- AND EXTRA-PAIR MATING SUCCESS IN THE AMERICAN REDSTART

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Abstract. This study examines the relationship of carotenoid-based plumage coloration to mating and reproductive success in a migratory songbird, the American Redstart (*Setophaga ruticilla*). Adult male redstarts have several highly variable orange carotenoid-based patches of plumage on their wings, flanks, and tail. We tested the prediction that males with larger and more intensely orange plumage patches have higher pairing success, as well as higher within-pair, extra-pair, and total reproductive success. We also quantified nestling-provisioning rates, to determine if carotenoid-based coloration was related to males' provisioning behavior. Males that paired successfully had significantly brighter orange flanks than unmated males. Paternity testing with microsatellites showed that 64% of broods (18 of 28) had extra-pair young and 44% of nestlings (36 of 81) were extra-pair. We found no relationship between carotenoid-based coloration and within- or extra-pair paternity. Contrary to our predictions, males with less saturated orange flanks sired more total young than more saturated males. Males with brighter orange flanks also provisioned nestlings at rates lower than did less bright males. These unexpected results highlight the need for further research on the mechanisms governing the production and maintenance of carotenoid-based signals in redstarts, as well as incorporating the ecology of migratory species into the study of carotenoid-based signals, which heretofore has been based primarily on nonmigratory species.

Key words: American Redstart, breeding success, carotenoid, coloration, extra-pair paternity, pairing success, pigmentation, Setophaga ruticilla.

Relación entre la Coloración Basada en Carotenoides y el Éxito de Apareamiento y Reproducción Intrapareja y Extrapareja en *Setophaga ruticilla*

Resumen. Este estudio examina la relación entre la coloración del plumaje basada en carotenoides y el éxito de apareamiento y reproducción en *Setophaga ruticilla*, un ave canora migratoria. Los machos de esta especie presentan varios parches altamente variables de color naranja basados en carotenoides en sus alas, flancos y cola. Pusimos a prueba la predicción de que los machos con parches de plumaje más grandes y de color naranja más intenso presentan un mayor éxito de apareamiento, así como un mayor éxito reproductivo intrapareja, extrapareja y total. También cuantificamos la tasa de alimentación a los pichones para determinar si la coloración basada en carotenoides se relacionaba con el comportamiento de aprovisionamiento los machos. Los machos que se aparearon exitosamente presentaron flancos de color naranja significativamente más brillantes que los machos que no consiguieron pareja. Los análisis de paternidad realizados mediante microsatélites mostraron que el 64% de las parvadas (18 de 28) tenían crías extra-pareja y que el 44% de los pichones (36 de 81) eran extra-pareja. No encontramos relación entre la coloración basada en carotenoides con la paternidad intrapareja ni extrapareja. En contraste con nuestras predicciones, los machos con flancos de color naranja menos saturado produjeron más crías en total que los machos con parches de color más saturado. Además, los machos con flancos naranja más brillantes presentaron tasas de aprovisionamiento a los pichones menores que los machos menos brillantes. Estos resultados inesperados resaltan la necesidad de más investigaciones sobre los mecanismos que controlan la producción y el mantenimiento de las señales basadas en carotenoides en *S. ruticilla*. Además, es importante incorporar la ecología de las especies migratorias en los estudios de las señales basadas en carotenoides, los cuales se han basado principalmente en especies no migratorias hasta ahora.

INTRODUCTION

Sexual selection is regarded as one of the most important factors in the evolution of extravagant coloration and plumage displays, particularly sexually dimorphic traits (Darwin 1871). Sexual-selection theory predicts that individuals with the most extravagant ornaments (e.g., brightest or largest pigmented area) should have the highest mating success (Fisher 1930, Zahavi 1975, Hamilton and Zuk 1982, Kodric-Brown and Brown 1984). Carotenoid-based plumage signals are

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common in birds and have been positively linked to choice of social and extra-pair mates, males having the largest or most elaborate patches of ornamental carotenoid pigmentation being preferred as social mates and/or more successful in reproduction (see Hill 1990, Sundberg and Dixon 1996, Wolfenbarger 1999). Ornamental pigmentation has been correlated with many measures of male quality, including individual health and fitness (Hill 1990, Gray 1996, Jawor and Breitwisch 2004), dominance and/or ability to defend resources (Pryke et al. 2002), resistance to pathogens and/or parasites (Hamilton and Zuk 1982, Brawner et al. 2000), and level of parental care (Hill 1991, Jawor and Breitwisch 2004), supporting earlier hypotheses that females may gain direct and/or indirect benefits from choosing colorful males as mates (see Endler 1980, Lozano 1994).

The extent to which females should choose mates by plumage cues depends on whether plumage color is a good predictor of a male's condition or genetic quality. Since birds are unable to synthesize carotenoid pigments from basic biological precursors (Fox 1976, Goodwin 1984), the expression of carotenoid-based coloration is linked to the ability of an individual to acquire carotenoid pigments from its diet (Brush and Power 1976, Brush 1978, Slagsvold and Lifjeld 1985) and its allocation of circulating and stored carotenoids to feathers rather than to physiological and immune functions (McGraw and Ardia 2003). Experiments that limited birds' access to food or increased their parasite load found that the limitation reduced the extent or content of carotenoids in the plumage (McGraw and Hill 2000, McGraw et al. 2005). Thus carotenoid signals relay reliable information about a male's feeding ability, body condition, and/or resistance to disease and parasites. Carotenoid-based plumage influences social mate choice, females preferring to mate with more ornamented or colorful males (Hill 1990, Hill et al.1994, Johnson et al. 1993, Sundberg 1995). However, the few studies relating extra-pair mate choice to carotenoid-based plumage have found that less-ornamented males are not more likely to be cuckolded (Hill et al. 1994, Sundberg and Dixon 1996).

This study investigates the relationship between carotenoid-based plumage coloration and the social and genetic mating success in a migratory songbird, the American Redstart (*Setophaga ruticilla*). The American Redstart is a small (9 g), territorial, sexually dimorphic, socially monogamous songbird in which extra-pair paternity is frequent (Perreault et al. 1997, Chiver et al. 2008). On their wings, tails, and flanks, males have several highly variable patches, ranging in color from yellow to orange-red (Norris et al. 2004), that are pigmented by carotenoids acquired from their diet (McGraw 2006). The male's predominant courtship display is the bow, in which males draw attention to these patches by holding the head up while tipping the breast toward the ground, spreading the tail, and pointing the wings upward and outward,

waving them at the female (Ficken 1963; PJK pers. obs.). Norris et al. (2004) discovered that the color of these patches is related to male reproductive effort: males with high reproductive effort subsequently grew yellowish tail feathers less saturated in color. Reudink et al. (2009) found that a male's coloration on arrival at the wintering grounds predicted the quality of its winter territory; males with brighter tail feathers held higher-quality territories. In addition, males that held high-quality winter territories had brighter tail feathers when they arrived at the breeding grounds (Reudink et al. 2009).

We predicted that carotenoid-based ornamental plumage should be positively correlated with pairing success, within- and extra-pair paternity, and total reproductive success. To test these predictions, we quantified the size and reflectance of the orange patches on the wings, tail, and flanks of males. We then determined if males' color characteristics were related to success in attracting a social mate and tested paternity by microsatellite analysis to determine males' within-pair, extra-pair, and total reproductive success. We also measured males' feeding rates to evaluate whether females gain direct benefits from pairing with more ornamented males.

METHODS

FIELD METHODS

We conducted this research from May to July 2002 and 2003 at Hemlock Hill Biological Research Area, Crawford County, Pennsylvania (41° 46′ N, 79° 56′ W). The study site is approximately 150 ha and vegetated primarily with mature mixed-hardwood forest surrounded by predominantly agricultural land. The study site supports over 100 breeding pairs of redstarts; we focused our study on a core area with 30 breeding pairs. We captured birds by using mist nets and playback of male song and banded all adult birds with an aluminum U.S. Fish and Wildlife Service band and a unique combination of three color bands. Dates of captures ranged from 5 May to 16 July (2002) and 3 May to 17 July (2003). Orange and red bands were not used. We measured the tarsus, wing length, and mass of each bird and collected a small blood sample (25–100 µl) via brachial puncture. In the American Redstart plumage maturation is delayed: males do not achieve adult plumage until their third year (Sherry and Holmes 1997). Because in our population territorial second-year males were so few (5%), we did not include them in our analyses.

We used several measures to determine whether or not a male paired successfully. In both years, from 1 May to 20 July, we observed behavior within the core study area daily to confirm individual males' territories. Watches extend from as early as 15 min before sunrise to as late as 17:30. If we found an active nest (e.g., during construction, incubation, or the nestling period) on a male's territory, we considered that individual paired. All males observed delivering food to a

female (typically on the nest), nestling, or fledgling were also considered paired. To be conservative, we categorized males that never had an active nest on their territory, were never seen delivering food to a female or young but nevertheless were seen associating with a female (e.g., courtship displays, prospecting for nest sights) at least once as unknown for pairing status; we excluded them from the pairing models (yes/no). Males that never had an active nest on their territory and were never seen delivering food to a female or young were considered unpaired.

We searched territories of mated males and over the study located roughly 200 nests. Nests were typically 1–12 m above the ground in trees and saplings. We monitored nests every 2 to 3 days to determine nest initiation, hatch date, clutch size, and fledging success. If a nest was accessible, we banded and obtained a blood sample (25 µl) from each nestling when the nestlings were at least 5 days old. In 2002 nest predation was over 80%, preventing us from obtaining paternity data for the offspring of many pairs.

Parental feeding rates were monitored during early (nestling age 4–5 days), mid (6–7 days), and late (8–10 days) stages, An 8-mm Sony digital camera mounted on a tripod was positioned >5 m from each nest at least 1 hr before recording began to allow adults to become habituated to it. Feeding trips to the nest were recorded for 2 hr during each of the three sessions (early, mid, late), beginning as early as 07:00 and ending as late as 19:00. Video footage was later transcribed to determine male and female feeding rates.

QUANTIFYING PLUMAGE COLORATION

To assess the size of the patches of carotenoid-pigmented plumage on the wings and tail, we extended the wings and tails of captured birds to allow full viewing of all orange feathers. We used an 8-mm Sony digital video camera set at a standard distance of 25 cm to record the plumage. A wing-chord ruler was placed in the frame during all videography to allow for later measurement of the ornaments.

We imported the digital video into the software program iMovie and saved individual frames as still pictures (see Thusius et al. 2001). Using Image J 1.28, PJK estimated the size of the carotenoid ornaments by tracing their outlines, after scaling the image with the ruler present in all videos. For each male we analyzed three different images of its wings and tail so that we could test the repeatability of the measurements. We used the average of the three measurements of each male's ornament for subsequent analysis. For the wing patch we summed the average size of the left and right sides. Measurement error, calculated as the percentage of total error of measurements of one individual (Yezerinac et al. 1992), was ≤0.69% for the wing patches; these measurements were highly repeatable ($r = 0.99$; Lessells and Boag 1987). Measurements of the size of the tail patches were not repeatable ($r = 0.39$) so were not used in the analyses.

To quantify the hue, saturation, and brightness of carotenoid ornaments, we recorded spectra of the flanks, wings, and tail in the field with a Digital Swatchbook spectrometer (X-rite, Inc., Grandville, MI), 400–700 nm. The orange of the flanks, wings, and tail of the American Redstart does reflect in the ultraviolet range (300–400 nm; McNett and Marchetti 2005), but our goal was to examine the highly variable amount of saturation (Norris et al. 2004, Reudink et al. 2009). All reflectance spectra were generated in proportion to the Swatchbook's white reference standard, and the spectrometer was calibrated before each bird was measured. When measuring the tail region, PJK wore a black fleece glove on the hand placed behind the tail feathers. We measured the spectra of each of the three body regions five times, arbitrarily placing the illuminating eye within the area of interest each time. Using the program Colorshop, we averaged these five replicate spectra for each body region of each male (Fig. 1).

We analyzed three components of the averaged spectral data: hue, saturation (spectral purity), and brightness (spectral intensity). Hue, referred to here as orangeness (for carotenoid-based coloration), was calculated as the wavelength at which reflectance (R) is 0halfway between its maximum and minimum ($\lambda_{R\mathrm{mid}} = [R_{\max} + R_{\min}]/2$; (Andersson et al. 2002, Montgomerie 2006). We used the value $\lambda[R50]$ to separate the spectral curve into two segments and used the difference of these two segments divided by the total reflectance ($R_{400-\lambda[R50]} - R_{\lambda[R50]-700})/R_{400-700}$), to calculate saturation (by this formula plumage of a more saturated color has a more negative value). Brightness was estimated as the total reflectance between 400 and 700 nm ($R_{400-700}$).

PATERNITY ANALYSIS

Blood samples were stored at 4 °C in 500 µl of Queen's lysis buffer (Seutin et al. 1991). We extracted DNA from the blood by incubating a subsample in cell-lysis buffer at 60 °C and then added ammonium acetate and isopropanol to precipitate DNA (L. De Sousa, unpubl.protocol) or used Qiagen DNEasy Kit (Qiagen, Mississauga, ON). We then resuspended the DNA in 200 µL of TE (10 mM Tris–HCl, 0.2 mM EDTA, pH 7.5).

We genotyped each sample at three variable microsatellite loci (*Dpμ01*, *Dpμ15*, *Dpμ16*) isolated from the Yellow Warbler (*Dendroica petechia*) (Dawson et al. 1997) and at one locus (*Cuμ04*) isolated from the Swainson's Thrush (*Catharus ustulatus*) (Gibbs et al. 1999). To assess variation among individuals for specific loci, we performed polymerase chain reactions (PCR) in 10-µL volumes that contained 2.5 µL of resuspended genomic DNA, 1.0 µL of forward primer labeled with ^{33}P-dATP, 1.0 µL of unlabeled forward primer, 2.0 µL of unlabeled reverse primer, 0.5 µL dNTPs, 0.1 µL Taq, 1.0 µL buffer, and 2.0–3.0 µL MgCl$_2$. PCR cycling consisted of an initial denaturing step at 94 °C for 3 min and 30 cycles consisting of 30 sec at the annealing temperature and 30 sec at 72 °C. PCR products were resolved on a 6% denaturing
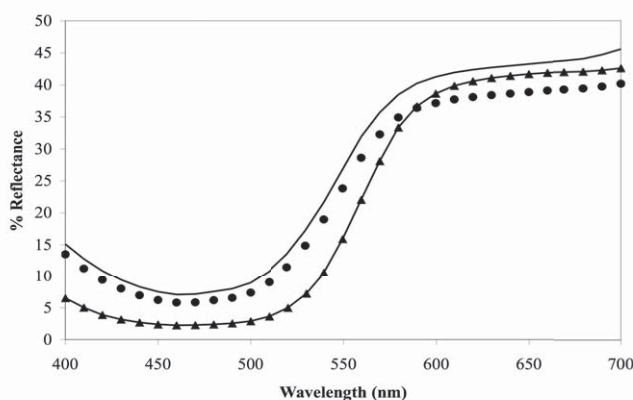
FIGURE 1. Representative reflectance spectra calculated from the average reflectance curves for the wing patch (circles), flank patch (triangles with solid line), and tail patch (solid line) of male American Redstarts. Reflectance spectra are expressed as the proportion of light reflected relative to a 100% white standard.

polyacrylamide gel and visualized with autoradiograph film. We determined the size of each allele by comparing PCR fragments to known-size reference samples run on each gel. All four loci were highly variable (16–29 alleles), with high levels of observed heterozygosity and high probabilities of exclusion. The cumulative exclusion probability [$P(E)$ (Chakraborty et al. 1988)] of the four loci was $P(E) < 0.0001$, indicating that we could identify extra-pair young and their sires with a high degree of certainty.

To determine the identity of the genetic parents we compared all possible adult–offspring allele combinations at each locus. Loci at which a putative parent and nestling differed by more than two base pairs were considered to be a mismatch. Loci that differed by two or fewer base pairs were considered a successful match, as the differences were likely laboratory artifacts (e.g., PCR amplification error or template–primer mismatch) or mutations. Nestlings who mismatched their social father at one or more loci were considered to be extra-pair young. Extra-pair sires were assigned parentage if they matched a nestling at all loci.

Eleven broods were excluded from further analysis of parentage assignment because of a mismatch between the social mother and one or more of the nestlings ($n = 7$) or the social father was poly-territorial and/or socially polygynous ($n = 4$). We identified social parents from field observations of nest defense and nestling feeding.

We assessed male reproductive success by measuring within-pair paternity (the number of young sired by a male in his own nest), extra-pair paternity (the number of young sired in nests of females other than the social mate's), and total reproductive success (the total number of young sired). This estimate of reproductive success excludes males that never had nestlings because their nests were depredated as well as those

nestlings that were sampled for DNA but were subsequently preyed on.

STATISTICAL ANALYSES

We performed Kolmogrov–Smirnov tests of normality on all variables when sample sizes were >50 or Shapiro–Wilk tests when sample sizes were ≤50. None of the variables in this study deviated significantly from normality, so no transformations were needed for parametric analysis. Color data (hue, saturation, and brightness) from the three plumage patches (flank, tail, and wing) were compared with analysis of variance (ANOVA). Because the colors of each patch differed, we analyzed the results for each patch separately. To determine which color variable(s) best predict measures of male reproductive success, we constructed three backward stepwise multiple-regression models for each measure of male reproductive success for each plumage patch, for a total of nine models, incorporating each component of color, year, capture date, the size of the orange wing patch, and body size as independent variables. We used the residuals of mass on tarsus length to estimate body size. We followed a backward selection procedure so that variables whose predictions of male traits were significant in combination would be included in the models even if they were not significant predictors individually (Zar 1999). We used a stepwise selection procedure because we believed important relationships among color components and plumage patches had not been thoroughly described, important covariates were likely still unknown, and associations among color components, plumage patches, and social and genetic mating success had not been described in the American Redstart. Under these conditions, stepwise selection procedures provide a fast and effective means to screen a number of variables and assess their associations with a given outcome (Hosmer and Lemeshow 2000). Among statistical tests, the sample sizes differ because not all ornaments were measured on all individuals, for some family groups not all chicks were genotyped, and not all nests were monitored for feeding rates. All values reported are mean ± SE unless stated otherwise, and all probabilities are two-tailed. Data were analyzed with SPSS 11.0 (SPSS 2003).

RESULTS

VARIATION IN BODY SITE, PATCH SIZE, AND PLUMAGE REFLECTANCE

In this population the size of the males' orange wing patch was 651 ± 16 mm^2 ($n = 94$) and was highly variable (range 354–1042 mm^2). The size of the patch, however, was not correlated with the length of the wing or body size (all $P \geq 0.07$), and the average body size and size of the wing patch in the two years did not differ (all $P \geq 0.40$). In both years, wing-patch size and body size of individuals captured did not differ significantly (all $P \geq 0.25$), although the sample size was small ($n = 13$), resulting in

TABLE 1.   Correlation matrix of the hue (H), saturation (S), and brightness (B) of the orange plumage on the flanks, tail, and wings of male American Redstarts ($n = 95$). Correlation coefficients (r) are above, P-values are below.

	Flank			Tail			Wing		
	H	S	B	H	S	B	H	S	B
Flank									
H									
S	−0.58								
	<0.01[a]								
B	−0.05	−0.05							
	0.65	0.65							
Tail									
H	0.32	0.03	0.06						
	<0.01[a]	0.75	0.55						
S	0.27	0.27	−0.07	−0.34					
	0.008	0.007	0.52	<0.01[a]					
B	−0.07	0.004	0.09	−0.12	0.05				
	0.50	0.97	0.38	0.25	0.63				
Wing									
H	0.37	0.12	−0.19	0.55	−0.04	−0.20			
	<0.01[a]	0.27	0.07	<0.01[a]	0.70	0.06			
S	−0.06	0.13	−0.08	−0.07	0.31	−0.08	−0.37		
	0.55	0.20	0.43	0.50	<0.01[a]	0.42	<0.01[a]		
B	−0.10	0.01	0.25	−0.14	−0.01	0.10	−0.40	0.29	
	0.35	0.93	0.02	0.18	0.91	0.33	<0.01[a]	<0.01[a]	

[a] P-values significant by the Bonferonni method, which sets a significance level of $P = 0.006$ for 9 comparisons.

only moderate power to detect age effects. The hue of the orange wing patch was negatively correlated with body size ($r = -0.34$, $P = 0.001$), and the saturation was positively correlated with body size ($r = 0.25$, $P = 0.01$), meaning the wing patches of larger birds were less saturated and more yellow-orange.

While there were some significant correlations between color components of the three plumage patches, there were no consistent trends (e.g., hue of the flank, tail, and wing were correlated; Table 1). One-way ANOVAs with Bonferroni tests for multiple comparisons revealed that the flanks were a more saturated orange-red hue than the orange patches of the tail and wing (Table 2). In addition, the orange of the flank, tail, and wings all differed significantly in brightness, the tail

feathers being the brightest and the flank patch the least bright (Table 2). Independent t-tests found that the saturation of the wings varied by year (2002: -0.57 ± 0.005; 2003: -0.59 ± 0.005; $t = 3.24$; $P = 0.002$), as did brightness of the flanks (2002: 6.30 ± 0.15; 2003: 6.69 ± 0.10; $t = -2.24$; $P = 0.03$) and tail (2002: 7.89 ± 0.15; 2003: 8.34 ± 0.08; $t = -2.92$; $P = 0.004$). On average, males captured in 2003 had wing patches more orange (i.e., saturation values more negative) and flanks and tail patches brighter than those of males captured in 2002. Individuals captured in both years had more saturated orange wing patches in 2003 ($\bar{x} = -0.65 \pm 0.009$) than in 2002 ($\bar{x} = -0.60 \pm 0.006$; paired t-tests: $t_{12} = 2.69$, $P = 0.02$) but differed by year in no other color components (all $P \geq 0.054$).

TABLE 2.   Results of a one-way ANOVA of hue, saturation, and brightness values (mean $\pm$ SE) calculated from the reflectance spectra of the orange patches on the flanks, tail, and wings of male American Redstarts ($n = 95$).

Color component	Flank	Tail	Wing	F	P
Hue	560.27 ± 0.45[a]	548.65 ± 0.40	548.27 ± 0.29	308.79	<0.001
Saturation	-0.72 ± 0.005[a]	-0.57 ± 0.005	-0.58 ± 0.004	306.96	<0.001
Brightness	6.52 ± 0.09[a]	8.14 ± 0.08[a]	7.24 ± 0.07[a]	101.79	<0.001

[a] Mean difference of values significant at the 0.05 level of post hoc Bonferonni tests for multiple comparisons.
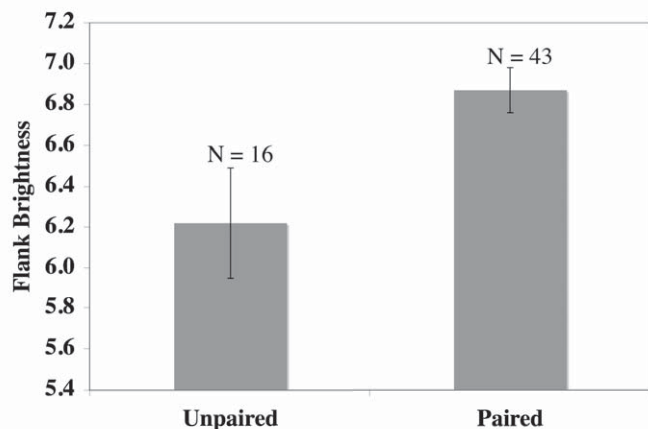
FIGURE 2. The relationship between pairing success and flank brightness of male American Redstarts; paired males ($n = 43$) had flank patches brighter ($\bar{x} = 6.78 \pm 0.11$) than did unpaired males ($n = 16$; $\bar{x} = 6.22 \pm 0.27$; independent-samples t-test: $t = -2.27$, $P = 0.03$).

PLUMAGE CORRELATES OF PAIRING SUCCESS

Of the 59 males whose pairing status during the study was known, 16 (27%) did not obtain a social mate. Males that successfully attracted a social mate had significantly brighter flanks than did unpaired males (Fig. 2) but did not differ significantly from unpaired males in body size, the size of their wing patch, or other color components (independent samples t-test: all $P \geq 0.23$). The data had sufficient power (0.80) to detect moderate differences between groups, and the single significant difference in flank brightness should be interpreted with caution because of the number of multiple comparisons performed.

PLUMAGE CORRELATES OF PATERNITY

Overall, 36 of 81 (44%) young resulted from extra-pair mating in 18 of 28 (64%) broods (Table 3). Nest predation approached 85% in 2002, resulting in an unexpectedly small sample of family groups that year ($n = 7$ nests). Social males sired an average of 1.6 ± 1.4 young ($n = 28$) out of an average of 2.9 ± 1.1 young per nest.

Bivariate correlations revealed no significant relationships between the number of within-pair young and wing-patch size, wing chord, or any component of carotenoid coloration of the flank, tail, or wing (all $P \geq 0.14$). In a backward stepwise multiple-regression analysis of the flank, tail, or wing (all $P \geq 0.13$), components of carotenoid coloration did not predict the number of within-pair young a male sired.

Using genetic parentage analysis, we were able to assign 14 of 36 (39%) extra-pair offspring in eight separate broods to 10 different extra-pair males (Table 3). Three of the males identified as extra-pair sires were not socially paired. The number of extra-pair young was not correlated with male body size, size of wing patch, or any color component (all $P \geq 0.08$). In addition, the carotenoid color of males that sired extra-pair young did not differ significantly from that of males that did not sire any extra-pair young (independent sample t-tests: all $P \geq 0.06$). We compared characteristics of extra-pair fathers and the males that they cuckolded and found no significant differences in the components of carotenoid-based coloration or other physical characteristics (paired t-tests: all $P \geq 0.06$), although the sample size was small ($n = 7$), resulting in low power to detect differences.

The total reproductive success of males that sired extra-pair young (1.8 ± 1.3 young) did not differ significantly from that of males that sired no extra-pair young (1.8 ± 1.4; independent t-test: $t_{30} = 0.05$, $P = 0.96$). Total reproductive success (measured as the total number of within-pair plus extra-pair young sired) averaged 1.8 ± 1.4 over the two seasons (range 0–5, $n = 32$ males). Bivariate correlations showed that flank saturation was significantly correlated with the total number of young a male sired ($r = 0.47$, $P = 0.008$, $n = 31$) but showed no other relationships between any of the other components of carotenoid-based plumage ornamentation and the total number of young sired (all $P \geq 0.11$). Separate backward stepwise multiple-regression models of the tail and wing plumage showed that while body size, year, wing-patch area, and color components did not predict the total number of young a male sired, flank saturation did predict the total number of young a male sired ($r^2 = 0.22$, $F_{30} = 8.21$, $P = 0.008$; Figure 3), with males with flanks less saturated in color siring more young.

TABLE 3. Extra-pair paternity of American Redstarts tested.

|	2002	2003	Total
Number of nests analyzed	7	21	28
Percentage nests containing extra-pair young	57	67	64
Number of chicks sampled	19	62	81
Percentage of extra-pair young	32	48	44
Percentage of extra-pair young per nest (mean ± SD)[a]	20.75 ± 24.95	54.08 ± 37.52	45.75 ± 35.42
Percentage of extra-pair young with sire identified	67	33	39
Percentage of nests with >1 extra-pair sire	67	80	75

[a] Nests with ≥3 nestlings.
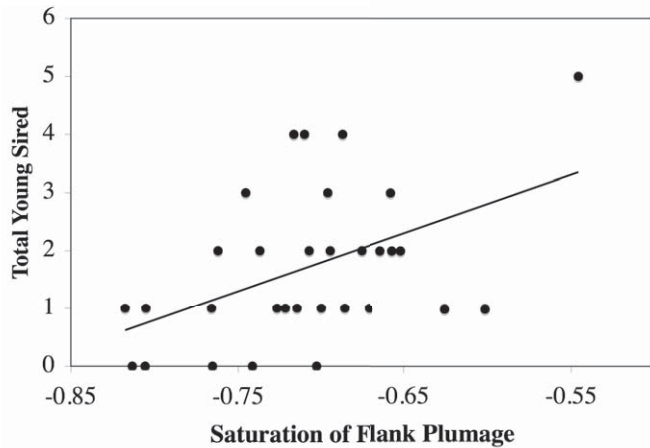
FIGURE 3.   The relationship between saturation of flank plumage and the total number (within-pair plus extra-pair) of young sired. More negative values correspond with more saturated plumage; regression line shown.
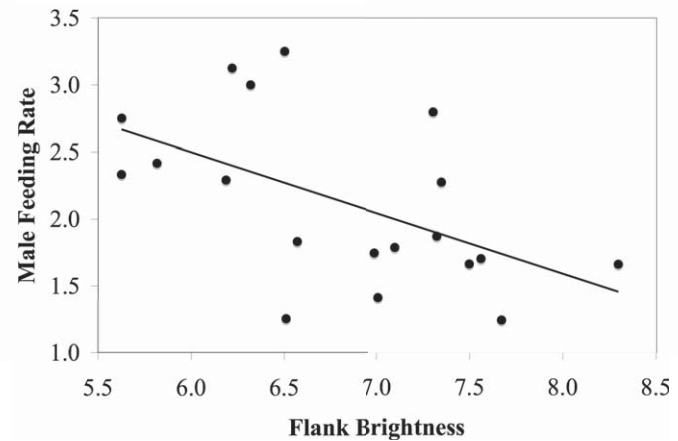

FIGURE 4.   The relationship between flank brightness and males' provisioning rate (deliveries per chick per hour); males with brighter flanks make fewer deliveries per chick per hour; regression line shown.

NESTLING-PROVISIONING RATES AND PLUMAGE COLORATION

To assess possible direct benefits that females gain from their social mates, we analyzed whether males' feeding rate was positively correlated with carotenoid ornamentation. Males fed nestlings (2.11 ± 0.14 visits chick^{-1} hr^{-1}) at the same rate as females (2.22 ± 0.22 visits chick^{-1} hr^{-1}; paired t-test: $t = -0.45$, $P = 0.66$, $n = 20$). Males' feeding rate was negatively correlated with the brightness of the orange on the flanks ($r = -0.53$, $P = 0.015$, $n = 20$; Fig. 4) but was not correlated with any other color component of the flanks, tail, or wing (all $P \geq 0.15$).

DISCUSSION

Male American Redstarts that paired socially had significantly brighter orange flank patches than unpaired males. Pairing success has been positively linked to carotenoid-based coloration in the American Goldfinch (*Carduelis tristis*; MacDougall and Montgomerie 2003), Golden-collared Manakin (*Manacus vitellinus*; Stein and Uy 2006), House Finch (*Carpodacus mexicanus*; Hill 1990, Hill et al. 1999, Badyaev and Hill 2002), Northern Cardinal (*Cardinalis cardinalis*; Jawor et al. 2003), and Village Weaver (*Ploceus cucullatus*; Collias et al. 1979), but this study is the first to find a relationship between a component of males' carotenoid-based plumage coloration and pairing success in the American Redstart. The positive relationship between plumage brightness and pairing success we found in this study and that Stein and Uy (2006) found in the Golden-collared Manakin is difficult to interpret because feathers high in carotenoids have higher hue values (i.e., shifted towards the red end of the spectrum) and higher saturation values but are less bright (Andersson and Prager 2006), so females might be expected to prefer males with less bright feathers.

One possible explanation is that the ultraviolet component of the carotenoid-based color is actually responsible for the relationship between brightness and pairing success. The carotenoid-based plumage patches that we measured in the American Redstart have a secondary reflectance peak in the ultraviolet (300–400 nm; McNett and Marchetti 2005, Reudink et al. 2009). Ultraviolet coloration is related to feather structure (Eaton and Lanyon 2003), and experimental removal of the structural component of carotenoid-based plumage results in duller feathers (Shawkey and Hill 2005), indicating the importance of structure to the expression of brightness in some carotenoid-pigmented feathers. In the Golden-collared Manakin, Stein and Uy (2006) found that the ultraviolet hue of the collar was correlated with collar brightness, suggesting that brightness signals a male's condition via the structural component of carotenoid-based signals. Further research on the biochemical and nanostructural mechanisms responsible for orange-red coloration (Stein and Uy 2006) is needed for interpretation of the many aspects of visual signals available to females viewing a male's plumage.

Alternatively, plumage brightness may not be a signal to females of a male's condition but rather a dominance-based status signal. In the American Redstart, Reudink et al. (2009) found that brightness of the tail feathers predicted the subsequent quality of the nonbreeding territory on the wintering grounds in Jamaica. Furthermore, birds with brighter tail feathers displaced birds with less bright tail feathers from higher-quality nonbreeding habitat. Although their study did not investigate the direct relationship between dominance or fighting ability and quality of nonbreeding territory, Marra (2000) showed that dominance and aggression play a critical role in the redstart's acquisition of nonbreeding territory, and carotenoid-based coloration has been linked to dominance in the Red-collared Widowbird (*Euplectes ardens*; Pryke

et al. 2001, Andersson et al. 2002, Pryke et al. 2002). These findings indicate that carotenoid-based plumage, including plumage brightness, may signal fighting ability and may do so in redstarts. Females may benefit from pairing with socially dominant males, especially if these males control larger or higher-quality breeding territories or provide other direct benefits. Additional research is necessary to determine if plumage brightness does signal dominance in redstarts and plays a role in agonistic interactions between males on the breeding grounds.

Interestingly, Norris et al. (2004) found that male American Redstarts whose reproductive effort was high (both timing and amount of parental care taken into account) were more likely to molt during migration and to have feathers less saturated in color, indicating lower concentrations of carotenoids (Saks et al. 2003). Therefore, males making more reproductive effort should have brighter carotenoid-based plumage (Andersson and Prager 2006). In contrast, males with low reproductive effort, including failed breeders, molted prior to migration and had feathers with more saturated orange-red, meaning these males should be less bright (Andersson and Prager 2006). Carotenoid saturation of tail feathers varies geographically in the American Redstart, so males molting their tail feathers farther south during migration could have access to fewer carotenoids in their diet (Norris et al. 2007). It is also possible that the physiological stress of molting during migration may reduce carotenoid deposition (Hill 2000), though Norris et al. (2007) found that carotenoid coloration is not related to rate of feather growth, an indirect measure of body condition. This relationship raises the intriguing possibility that high reproductive effort one year may handicap a male's sexual signals, and hence mating success, the next (Hill 2004). We found, however, that males with brighter flanks had higher pairing success. If we continue to think of carotenoid-based coloration as a signal of body condition and the hue and saturation of feathers reliably indicate the timing of a male's molt, color could tell females something about the pattern of a male's breeding in the previous season. Males whose plumage is less saturated and lighter orange likely fledged young in the previous year, and females may therefore prefer these proven productive males as mates. Unfortunately, this line of reasoning fails to provide a mechanism for the maintenance of this signal. Presumably males whose reproductive success is lower should simply "cheat" and display less saturated plumage similar to that of males with high reproductive success, as there is no clear mechanism to prevent this deception.

However, if we think of plumage brightness as a signal of a male's competitive ability and aggression (regardless of the mechanisms that lead to increased brightness, e.g., ultraviolet component or less saturated colors), then a system for the maintenance of the honesty of this signal does exist. Males with high reproductive success produce less saturated (Norris et al. 2004) and brighter carotenoid-based plumage.

These males arrive on the wintering grounds with brighter plumage and occupy higher-quality habitats, returning to the breeding grounds brighter than birds that occupy lower-quality winter habitats (Reudink et al. 2009). These same males enjoy higher pairing success, and likely make the associated high reproductive effort, completing the cycle. Although we did not find a measure of brightness linked to reproductive effort, males that sired more total offspring did have less saturated flank plumage.

Relatively few studies have examined the role of carotenoid signals in extra-pair mate choice (see Griffith and Pryke 2006). Hill et al. (1994) found that drably pigmented male House Finches are no more likely to be cuckolded than brightly colored males. In the Yellowhammer (*Emberiza citrinella*) drably and brightly pigmented individuals are equally likely to be cuckolded (Sundberg and Dixon 1996). Brightly colored males sire more extra-pair young, but it is not known whether this resulted from female choice or differences in male–male competition and extra-pair behavior. In the Hooded Warbler (*Wilsonia citrina*) the yellow cheek patch and breast of males do not influence extra-pair mating success or the rate at which females visit neighboring males (Chiver et al. 2008). Likewise, we found that in the American Redstart carotenoid-based plumage did not influence within- or extra-pair mating success and that none of the color components of cuckolded males differed significantly from those of the extra-pair males that cuckolded them.

In an effort to assess if females gained direct benefits from their social partner, we quantified nestling-provisioning rates. Males with brighter flank plumage fed nestlings at a lower rate than did other males. Sundberg and Larsson (1994) found that older, more colorful Yellowhammers also provisioned nestlings at lower rates, and yet the number of young fledged was positively correlated with the male's color. In both cases, brighter or more colorful males could be delivering larger and/or higher-quality prey, to compensate for reduced rate of provisioning.

Most research on mate choice and carotenoid-based plumage has focused on the expression of carotenoid-based coloration as a signal of male condition and has relied heavily on seed-eating finches and sparrows (reviewed in Griffith et al. 2006), largely because such species are ideal subjects for laboratory experiments. Several studies, however, have examined the role of carotenoid-based plumage in social and extra-pair mate choice in insectivorous neotropical migrants, such as the American Redstart, and have found that more ornamented males do not have higher mating success (Parker et al. 2003, Tarof et al. 2005, Chiver et al. 2008) or, like ours, have revealed unexpected relationships that are difficult to explain via the hypothesis that carotenoid-based coloration signals body condition. For these species, males are territorial on the wintering grounds, colorful year round, and do not undergo an extensive spring molt (Froelich et al.

2005), unlike many finches and sparrows. The color of carotenoid-pigmented plumage grown in late summer, like that of the American Redstart, may be strongly affected by timing of breeding (Norris et al. 2004, Norris et al. 2007). The relation between molt and timing of breeding may influence the production of carotenoid-based coloration in novel ways, producing unexpected relationships between plumage coloration and what it signals to conspecifics. Studies of long-distance migrants that cover the entire annual cycle and address alternative hypotheses regarding carotenoid-based coloration may provide insights into the function and evolution of carotenoid-based ornamental traits.

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