



RESEARCH ARTICLE

Quantitative color variation within and across morphs of the polymorphic White-throated Sparrow

Nathan A. Rathbun,¹ Andrea S. Grunst,¹ Melissa L. Grunst,¹ Joanna K. Hubbard,² Rebecca J. Safran,² Rusty A. Gonser,¹ and Elaina M. Tuttle^{1*}

¹ Department of Biology, Indiana State University, Terre Haute, Indiana, USA

² Department of Ecology and Evolutionary Biology, University of Colorado at Boulder, Boulder, Colorado, USA

* Corresponding author: elaina.tuttle@indstate.edu

Submitted May 8, 2014; Accepted August 30, 2014; Published November 5, 2014

ABSTRACT

Coloration has evolved to serve diverse functions, including communication. In species with discrete color polymorphisms, the extent to which color variation exists within morphs and communicates multiple messages often remains unclear. We employed reflectance spectrometry to study variation in coloration in the dimorphic White-throated Sparrow (*Zonotrichia albicollis*), which exhibits a “white” and “tan” morph in both sexes. We explored whether distinct color traits distinguish between morph and sex classes, and whether color variation exists within classes that might reflect differences in individual quality. Further, we asked whether sexual dichromatism is more pronounced in the white morph, in which males display greater promiscuity and aggression and, thus, may be under stronger sexual selection for conspicuous coloration. Distinct aspects of crown plumage coloration differentiated the two morphs versus the two sexes and multiple types of coloration were associated with a morph, suggesting both multiple and redundant messaging functions of coloration. The brightness of white coloration and yellow carotenoid-based coloration differentiated the morphs, whereas the brightness and saturation of brown to black melanin-based pigmentation differentiated the sexes within morphs. However, coloration also varied considerably within morph and sex classes, potentially reflecting differences in individual quality. Finally, more sexual dichromatism existed within white morph than within tan morph birds. White morph males and females differed in white and yellow coloration, which also differentiated the morphs, and in melanin-based coloration. By contrast, tan morph males and females differed only marginally in coloration, and only in terms of melanin-based coloration. Results suggest that crown coloration is a multifaceted signal, and that selection has acted differently on coloration in both the morphs and the sexes. Our study suggests that multifaceted coloration can play multiple and redundant messaging functions, shows that color variation in polymorphic species can communicate more than morph, and suggests that morph-specific reproductive strategies alter selection on coloration.

Keywords: color polymorphism, color variation, sexual signaling, sexual dichromatism, White-throated Sparrows, *Zonotrichia albicollis*

Variación cuantitativa del color dentro y entre morfos de la especie polimórfica *Zonotrichia albicollis*

RESUMEN

La coloración ha evolucionado para servir a diferentes funciones, incluyendo la comunicación. En especies con polimorfismos de color discretos, la medida en que existe una variación de color dentro de los morfos y comunica varios mensajes a menudo sigue siendo poco clara. Empleamos espectrometría de reflectancia para estudiar la variación en la coloración en la especie dimórfica *Zonotrichia albicollis*, que exhibe un morfo “blanco” y “tostado” en ambos sexos. Exploramos si los rasgos distintivos de color permiten distinguir entre clases de morfo y sexo, y si la variación en color que existe dentro de las clases puede reflejar diferencias en calidad individual. Más aún, nos preguntamos si el dicromatismo sexual es más pronunciado en el morfo blanco, en el cual los machos despliegan más promiscuidad y agresión, y por lo tanto si las coloraciones conspicuas podrían estar bajo una presión de selección sexual más fuerte. Ciertos aspectos distintivos de la coloración del plumaje de la corona diferenciaron a los morfos versus los sexos, y múltiples tipos de coloración estuvieron asociados con el morfo, sugiriendo funciones de mensajería múltiples y redundantes por parte de la coloración. El brillo de la coloración blanca y la coloración amarilla basada en carotenoides diferenciaron los morfos, mientras que el brillo y la saturación de la pigmentación marrón y negra basada en melanina diferenció los sexos dentro de los morfos. Sin embargo, la coloración también varió considerablemente dentro de las clases de morfo y sexo, reflejando potencialmente diferencias en la calidad individual. Finalmente, existió más dicromatismo sexual dentro del morfo blanco que del morfo tostado de las aves. Los machos y las hembras del morfo blanco se diferenciaron en la coloración blanca y amarilla, que también diferenció a los morfos, y en la

coloración basada en melanina. En contraste, los machos y las hembras del morfo tostado se diferenciaron originalmente solo en la coloración, y sólo en cuanto a la coloración basada en melanina. Los resultados sugieren que la coloración de la corona es una señal multifacética y que la selección ha actuado diferencialmente sobre la coloración en los morfos y en los sexos. Nuestro estudio sugiere que la coloración multifacética puede jugar funciones de mensajería múltiples y redundantes, muestra que la variación del color en las especies polimórficas puede comunicar más que el morfo, y sugiere que las estrategias reproductivas específicas de los morfos alteran la selección basada en la coloración.

Palabras clave: plumaje, polimorfismo, variación del color, *Zonotrichia albicollis*

INTRODUCTION

Intraspecific and interspecific differences in coloration have evolved to serve a variety of communication functions, in addition to serving as camouflage (Darwin 1871, Andersson 1994). Sexual selection has shaped plumage coloration in many avian species, with coloration signaling phenotypic or genetic quality and acting to attract and retain mates (Hill 1991, 2002, McGraw 2003, 2006, Safran and McGraw 2004). Further, in addition to signaling individual quality differences, plumage pigmentation may communicate information about alternative reproductive strategies, as observed in some polymorphic species, including the Barn Owl (*Tyto alba*; Roulin et al. 2004), Red-footed Booby (*Sula sula*; Le Corre 1999), Ruff (*Philomachus pugnax*; Lank et al. 1995, Jukema and Piersma 2006), Gouldian Finch (*Erythrura gouldiae*; Pryke and Griffith 2007), Red-billed Quelea (*Quelea quelea*; Dale 2000), and White-throated Sparrow (*Zonotrichia albicollis*; Tuttle 1993, 2003). Territorial males may display bright plumage coloration that advertises their quality or fighting ability (Pryke and Griffith 2006). On the other hand, less aggressive males, such as satellite males and female mimics in lekking Ruffs, may display duller plumage to reduce aggression and increase the potential for sneak copulation (Tuttle 1993, Houtman and Falls 1994, Lank et al. 1995, Jukema and Piersma 2006). Plumage color in polymorphic species can be continuously distributed, as in Tawny Owls (*Strix aluco*; Brommer et al. 2005), or discrete, with little apparent variation in color within a morph (e.g., the Gouldian Finch; Pryke and Griffith 2007). In species with relatively discrete color polymorphisms, the extent to which variation in plumage coloration exists within morphs and signals meaningful individual differences is often unclear.

In species that display polymorphism in both sexes (e.g., Gouldian Finches and White-throated Sparrows), sexual dichromatism in plumage coloration may occur within morphs, and distinct aspects of coloration may differentiate the two sexes, as opposed to the two morphs. In many sexually dichromatic species, clear differences exist in the plumage traits of males and females, reflecting differences in selective pressures (Badyaev and Hill 2003). However, even in species in which plumage traits are not clearly distinct between males and females, subtle differences may

exist that serve to distinguish the sexes. Indeed, in avian species that appear monochromatic to human observers, spectrometric studies have revealed cryptic sexual dichromatism in the ultraviolet (UV) reflectance characteristics of plumage (Cuthill et al. 1999, Doucet et al. 2005, Burns and Shultz 2012). Signals of sex might be expected to evolve, given that discriminating between the sexes is essential to appropriate mate choice and aggressive interactions. Males may need to signal their identity to effectively defend breeding territories (Rohwer 1975, Siefferman and Hill 2005, Pryke and Griffith 2006), and females should signal their identity to avoid misdirected aggression from males (Coady and Dawson 2013). In addition, distinct selective pressures on males versus females may lead to discrepancies in plumage coloration, with sexual selection favoring brighter coloration in males, and natural selection against conspicuousness favoring duller coloration in females (Badyaev and Hill 2003).

To explore variation in plumage coloration within a polymorphic species and the potential for coloration to simultaneously convey information about morph and sex, we conducted a spectrometric study of plumage coloration in the White-throated Sparrow. The White-throated Sparrow has genetically determined plumage morphs that occur in both sexes and are associated with distinct reproductive strategies (Lowther 1961, Tuttle 1993, 2003). Color morph is genetically determined by a chromosomal polymorphism, which has arisen from a pericentric inversion of the second chromosome. White morph birds are heterozygous for chromosomes with the inversion, whereas tan morph birds are homozygous without the inversion (Lowther 1961, Thorneycroft 1966, 1975, Tuttle 1993, 2003, Thomas et al. 2008, Romanov et al. 2009). White morph males and females are more aggressive and provide less parental care to offspring than their tan morph counterparts (Tuttle 2003). Moreover, white morph males are often promiscuous and invest intensely in singing and in pursuit of extrapair copulations (Tuttle 2003, Horton et al. 2012). Both morphs display multifaceted crown coloration, with different components of coloration potentially playing different signaling roles. Specifically, individuals express a white throat and median crown stripe, brown to black lateral crown stripes, and yellow superciliary stripes (Figure 1). Although the biochemistry of pigmentation has not been directly

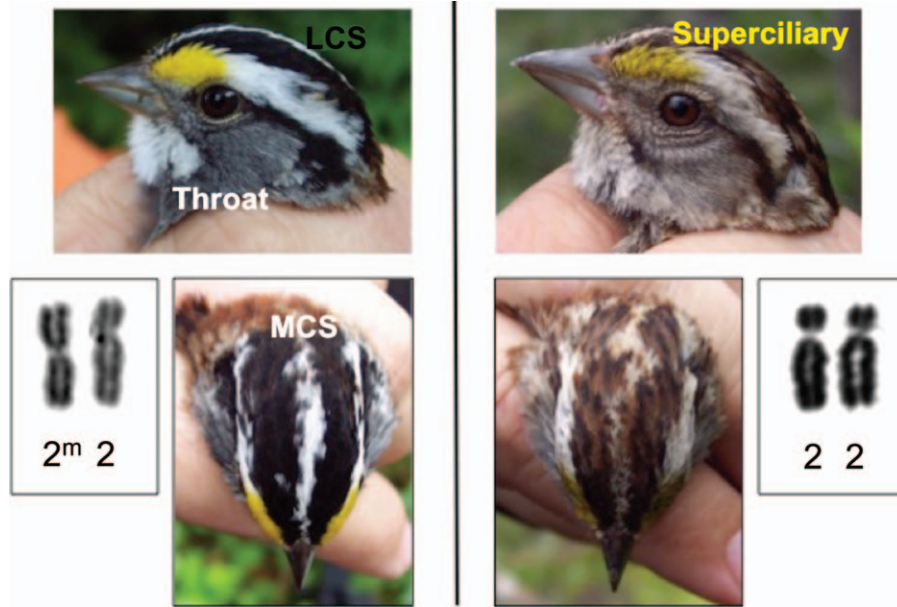


FIGURE 1. White morph (left) and tan morph (right) of the White-throated Sparrow, with location of the lateral crown stripe (LCS), median crown stripe (MCS), and supercilium indicated. White males exhibit a white median crown stripe, black lateral crown stripe, and bright yellow supercilium stripes. Tan males have a tan median crown stripe, more brown in the lateral crown stripe, and dull mustard supercilium stripes.

analyzed in the White-throated Sparrow, distinctive reflectance spectra indicate that melanins underlie brown to black coloration (McGraw et al. 2005), whereas yellow coloration is produced by carotenoids and white coloration is structural in origin (Andersson and Prager 2006). The 2 morphs of the White-throated Sparrow differ in crown plumage coloration (Knapton and Falls 1983, Kopachena and Falls 1991, 1993b, Tuttle 1993, 2003, Formica et al. 2004, Korody 2006, Tuttle et al. 2006; Figure 1), but the extent to which distinct patches of coloration differ between the morphs and the sexes within morphs has not been quantified previously.

We compared plumage coloration between the morphs and sexes across multiple feather patches and used discriminant function analysis to assess whether sex and morph classes—white male (WM), tan male (TM), white female (WF), and tan female (TF)—could be clearly distinguished via plumage coloration. Discriminant function analysis extracts the multidimensional axes that best distinguish between different classes of birds. Thus, using discriminant function analysis allowed us to assess whether different color traits play redundant (overlapping) roles in distinguishing morph and sex classes, or whether distinct combinations of color traits distinguish these groups, as might occur given a multiple-messages function of coloration. Further, we assessed whether variation in plumage coloration differed between morph and sex classes. To quantify plumage coloration, we used spectro-

metric measurements across the avian visual range, including UV wavelengths not visible to humans.

We made the following specific predictions. First, we predicted that significant differences in coloration exist between morph and sex classes, which birds could use to discriminate between these classes and respond appropriately. Indeed, in the White-throated Sparrow, the morphs mate disassortatively, which suggests that birds are capable of distinguishing the morph of their mate (Lowther 1961, Houtman and Falls 1994, Tuttle 2003). Furthermore, more promiscuous and aggressive white morph males present a greater threat to another male's fitness than tan males (Formica and Tuttle 2009), and discriminating the sexes is essential when determining whether to court a female or defend against another male. However, although the morphs are clearly visually distinct, behavioral rather than visual cues might serve to distinguish the sexes within the morphs, such that it might not be possible to consistently discriminate between the sexes via plumage coloration alone. In addition, given a multiple-messages function of complex coloration, we predicted that different aspects of crown coloration might distinguish the morphs versus the sexes. Further, although we did not quantify individual condition or fitness in the present study, certain components of coloration may not strongly distinguish morphs, if these color traits instead serve as condition-dependent signals of individual quality (Andersson 1994) and are thus highly variable within morphs. For instance, many studies show that carotenoid-based coloration (found in the

superciliary stripe in White-throated Sparrows) is related to individual condition. Carotenoids are in limited dietary supply and play roles in immune-stimulation and antioxidant defenses in addition to underlying coloration (McGraw 2006, Alonso-Alvarez et al. 2008). Thus, we predicted that carotenoid-based coloration would be highly variable within morphs and would be particularly variable within white morph males, which might be under strong sexual selection to signal their quality to prospective mates. Finally, we also predicted that sexual dichromatism and variation in plumage coloration might be greater within the white morph than within the tan morph. We made this prediction because promiscuous and highly territorial white morph males might be under stronger sexual selection for conspicuous plumage coloration than tan morph males. By contrast, because they provide more paternal care, tan morph males may be under selection against conspicuous plumage, which could attract predators to nests (Martin and Badyaev 1996).

METHODS

Analysis of Plumage Characteristics

We collected feathers from 107 adult White-throated Sparrows (42 white morph males, 16 white morph females, 32 tan morph males, and 17 tan morph females) during the breeding season (May–August, 2006–2009) at Cranberry Lake Biological Station (44°15'N, 74°48'W; Adirondacks, New York, USA). The study site consists of >32 ha of forest, pond, and bog. All birds on the study site are banded with a unique combination of colored plastic bands and a Fish and Wildlife band (Master Banding Permit 22296 to E. M. Tuttle). We captured birds via passive mist netting, as well as by song playback. We sampled feathers from the median crown stripe, lateral crown stripe, superciliary stripe, throat, back, and belly of each bird (Figure 1). We collected a minimum of 3 feathers from each patch, with 5 feathers sampled in the majority of cases. In the case of a few birds ($n = 6$), we did not sample all plumage patches or had fewer than 3 feathers from a particular patch. We excluded these birds from analyses involving that patch. To prevent pigment degradation, feathers were refrigerated and stored in the dark in 1.5-mL centrifuge tubes until spectrometric analysis.

We recognize that reflectance spectra obtained from feather samples may not be completely comparable to spectra obtained from plumage in situ, but we did not have access to a spectrometer at our field site. Thus, before obtaining reflectance spectra from feather samples, we did our best to recreate natural plumage configuration by carefully overlapping feathers, an approach that has frequently been adopted by previous researchers (Hörak et al. 2001, Freeman-Gallant et al. 2010, Grunst et al. 2014). We secured 3 to 5 overlapping feathers to blank

white index cards, using clear tape at the base of the feather. We used an average of 4 feathers per individual. We then collected reflectance data on each plumage patch, using a USB4000 Fiber Optic Spectrometer (Ocean Optics, Dunedin, Florida, USA) with Spectrasuite software (Ocean Optics). We positioned the probe vertically, 2.5 mm from the surface of the feather. We obtained 3 measurements from each patch from the exposed, colored part of the feather. We repositioned the probe between measurements to account for color variation within a patch. We analyzed reflectance data using the program CLR (version 1.05; Montgomerie 2008), which scored each feather patch for hue, saturation, and brightness. We used a wavelength range of 300–700 nm, corresponding to the avian visual range (Andersson and Prager 2006). Brightness (B) is defined as how much light is reflected at a given wavelength, and is equivalent to the area under the reflectance spectrum across the entire range of wavelengths. Mathematically, $B = \sum_{300}^{700} R_i$, where R_i is median reflectance at each integer wavelength (nm). Hue (H) indicates the dominant wavelength (color) reflected by the feather, as measured by a ratio between the amount of light reflected by different regions of the spectrum. Hue (H) was calculated using the formula $H = \arctan[(B_{\text{yellow}} - B_{\text{blue}})/B_{\text{total}}]/(B_{\text{red}} - B_{\text{green}})/B_{\text{total}}$, where blue = 400–475 nm, green = 475–550 nm, yellow = 550–625 nm, and red = 625–700 nm. Finally, saturation is indicative of the purity of coloration, with higher values of UV saturation indicating that more UV light is reflected, in relation to reflectance across the entire spectrum. For all feather patches except the superciliary, UV saturation (S) was calculated using the formula $S = \sum_{400}^{300} R_i/B$. Thus, less reflectance at visible wavelengths, due to greater deposition of melanins in feathers as well as more UV reflectance, increases UV saturation. For the carotenoid-bearing superciliaries, we calculated carotenoid saturation instead of UV saturation, using the formula $S_{\text{car}} = (\text{median } R_{700} - \text{median } R_{450})/(\text{median } R_{700})$. Greater carotenoid saturation is associated with greater purity of the carotenoid reflectance spectra, as accomplished through more deposition of carotenoids in plumage (Andersson and Prager 2006, Hegyi et al. 2007). Further, in the case of the White-throated Sparrow, greater carotenoid saturation is also associated with less masking of the carotenoid reflectance spectrum by deposition of melanins into superciliary feathers, which often appears to occur in tan morph birds (see Figure 1). For each patch of feathers, we averaged scores derived from the 3 reflectance spectra to obtain final metrics of plumage coloration.

Sex and Morph Determination

In the field, we determined the sex of each individual by noting the development of the cloacal protuberance (sperm storage organ) in males and the brood patch in

females, and morph using the visual criteria of Lowther (1961) and Piper and Wiley (1989). Further, we also verified individual morph and sex using molecular techniques. To obtain DNA, we took a blood sample (80–200 μ L) from the brachial vein and stored red blood cells in lysis buffer at 4°C (Longmire et al. 1992) until extracting DNA using the DNA IQ magnetic extraction system (Promega, Madison, Wisconsin, USA). We determined sex by using the P2 and P8 primers to amplify a conserved region of the CHD gene on the W and Z sex chromosomes (Ellegren 1996, Griffiths et al. 1998). We molecularly confirmed morph in the laboratory using a modification of the process described by Michopoulos et al. (2007). We found 100% agreement between field and molecular methods for assigning morph and sex.

Statistical Analysis

We conducted statistical analyses using SPSS version 16.0 (SPSS, Chicago, Illinois, USA) and R version 2.15.2 (R Core Team 2012). Because of unequal variances between groups, we used Kruskal-Wallis rank-sum tests to determine whether significant differences existed between the morph and sex classes with respect to each plumage coloration variable. When differences were identified, we proceeded to use pairwise Wilcoxon rank-sum tests with a Holm correction for multiple comparisons to assess which classes of birds significantly differed in coloration (Holm 1979).

To determine whether we could accurately place birds into morph and sex classes based on coloration, and to characterize the axes of variation that most effectively separated the classes, we used coloration variables that displayed significant differences between classes of birds in a discriminant function analysis in SPSS. Specifically, 9 crown coloration variables differed between the classes (see below), so these coloration variables were used in the discriminant function analysis. We did not use all coloration variables in the discriminant function analysis, to avoid using too many predictor variables in relation to our sample size. Although variance in coloration differed between groups, we used linear discriminant function analysis rather than quadratic discriminant function analysis, because a primary objective was characterizing how classes differed in coloration (an objective relatively robust to violation of the equality-of-variance assumption), and because quadratic discriminant analysis failed to improve classification success rate. We used Wilks's tests to assess the significance of linear discriminant functions and report loadings of discriminating (independent) variables on each discriminant function. Further, to clarify how the discriminant functions separated classes of birds, we used analysis of variance with Tukey's HSD post hoc tests or Kruskal-Wallis tests with post hoc Wilcoxon rank-sum tests (depending on whether normality and equality of

variance were realized) to compare mean class scores on the discriminant functions.

When classifying birds on the basis of the discriminant functions, we investigated classification success rates and patterns of misclassification, both when calculating prior probabilities based on proportions of classes present in the dataset and when setting prior probabilities equal at 25% each. We performed a chi-square test to determine whether we were able to predict group membership significantly more often than expected by chance alone. Finally, we used Levene's test (in R) to assess whether variance in coloration variables differed between the morph and sex classes. If the overall Levene's test for a given coloration variable returned a significant result, we performed separate tests to compare variance between classes of birds.

RESULTS

Characterization of Coloration Types

The different feather patches showed distinct reflectance characteristics, and reflectance spectra also showed distinct, qualitative differences between the morphs in several cases. In particular, the median crown stripe showed distinct reflectance spectra between the morphs of birds. Reflectance spectra from tan morph birds tended to be characteristic of melanin-based pigmentation, with reflectance slowly increasing across all wavelengths. By contrast, white morph birds with bright median crown stripe plumage appeared to have little melanin deposited in their median crown stripes. These birds displayed median crown stripe reflectance spectra more characteristic of structural reflectance. High reflectance occurred across most of the avian visual spectrum, including considerable reflectance in the UV wavelengths, as also seen for reflectance spectra taken from the white throat (Figure 2). The brown–black lateral crown stripe showed a reflectance spectrum characteristic of melanin-based pigmentation in all birds, with birds with very dark lateral crown stripes (generally white morph males) showing little lateral crown stripe reflectance at any wavelength (Figure 2). Finally, the yellow superciliaries displayed the characteristic reflectance spectrum of carotenoid pigmentation, with high absorbance at blue–green wavelengths (430–500 nm) and a peak in reflectance at both UV and yellow wavelengths (Figure 2; Britton et al. 1995, Keyser and Hill 1999, Andersson and Prager 2006). However, tan morph birds appeared to have melanins as well as carotenoids deposited in the superciliary region, resulting in a decrease in the brightness of superciliaries and some masking of the characteristic carotenoid reflectance curve in many tan morph birds (Figure 2). Finally, back feathers showed reflectance curves characteristic of melanin-based pigmentation, whereas the whitish belly feathers displayed

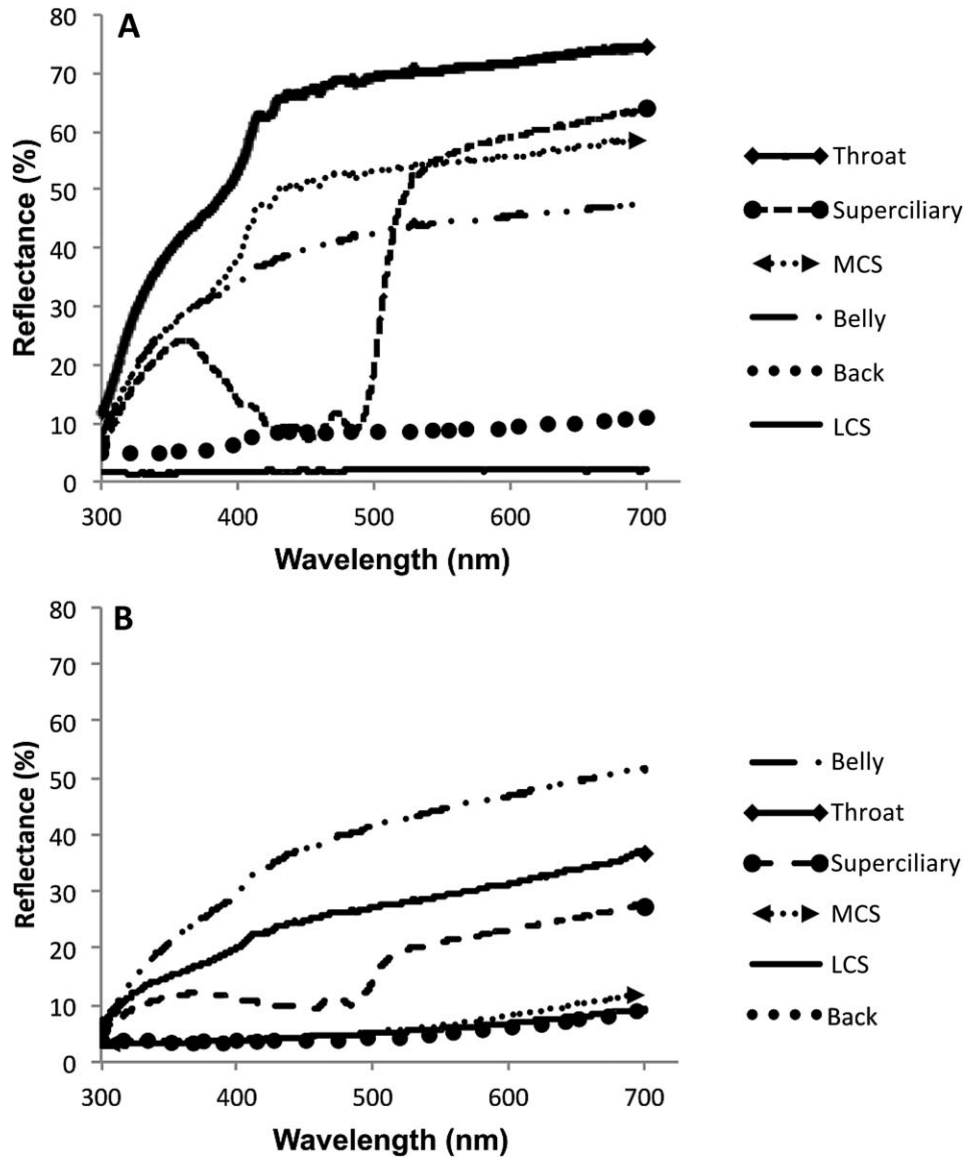


FIGURE 2. Examples of reflectance spectra from a bright white morph male (A) and a dull tan morph male (B). The white morph male displays a distinctly brighter median crown stripe (MCS), and also a bright throat and superciliary, but a darker (less bright) lateral crown stripe (LCS). In contrast to crown plumage patches, belly and back feathers show similar reflectance spectra between the 2 birds. To aid interpretation, the legend in each panel is arranged to reflect the vertical order in which reflectance spectra appear on the plots.

reflectance spectra more similar to the white throat. In Figure 2, we give examples of characteristic reflectance spectra from each patch of feathers from a bright white morph male and from a dull tan morph male.

Plumage Coloration Differences between Morph and Sex

The morph–sex classes of White-throated Sparrows showed distinct differences in plumage coloration with respect to the coloration of the crown and throat feathers, but did not differ significantly in the coloration of back or

belly feathers (Kruskal-Wallis rank-sum tests; Table 1). In pairwise post hoc comparisons, white morph males most frequently displayed significant differences in plumage coloration compared with other classes of birds. White morph males had brighter white median crown stripes (pairwise Wilcoxon rank-sum tests: $P < 0.01$) and greater median crown stipe hue values than all other classes of birds ($P < 0.01$), whereas none of the other classes differed significantly in median crown stipe coloration ($P > 0.10$). White morph males (but not white morph females) also had less bright (darker) lateral crown stripes than tan

TABLE 1. Kruskal-Wallis tests of differences in coloration between morph–sex classes and mean (\pm SE) values of color variables in each class. Abbreviations: TF = tan female, TM = tan male, WF = white female, and WM = white male, MCS = median crown stripe, and LCS = lateral crown stripe.

Color variable	χ^2	df	<i>P</i>	TF	TM	WF	WM
MCS brightness	41.53	3	<0.001	44.34 \pm 5.83	53.12 \pm 7.62	72.22 \pm 11.42	139.52 \pm 9.73
MCS saturation	1.15	3	0.764	0.14 \pm 0.006	0.14 \pm 0.003	0.15 \pm 0.005	0.15 \pm 0.003
MCS hue	38.69	3	<0.001	0.77 \pm 0.02	0.75 \pm 0.02	0.82 \pm 0.02	0.92 \pm 0.02
LCS brightness	16.52	3	<0.001	31.22 \pm 5.66	18.94 \pm 1.28	21.25 \pm 3.43	15.27 \pm 1.43
LCS saturation	26.62	3	<0.001	0.15 \pm 0.008	0.17 \pm 0.005	0.17 \pm 0.007	0.19 \pm 0.005
LCS hue	2.18	3	0.534	0.68 \pm 0.03	0.64 \pm 0.02	0.66 \pm 0.02	0.69 \pm 0.03
Superciliary brightness	42.47	3	<0.001	82.81 \pm 5.99	89.19 \pm 7.05	132.56 \pm 11.11	135.83 \pm 4.28
Superciliary saturation	26.21	3	<0.001	0.72 \pm 0.03	0.75 \pm 0.03	0.81 \pm 0.02	0.84 \pm 0.008
Superciliary hue	42.08	3	<0.001	0.96 \pm 0.01	0.96 \pm 0.02	1.03 \pm 0.02	1.03 \pm 0.005
Throat brightness	32.19	3	<0.001	191.29 \pm 10.01	182.09 \pm 8.61	227.60 \pm 7.56	236.84 \pm 5.04
Throat saturation	1.88	3	0.597	0.15 \pm 0.003	0.15 \pm 0.002	0.15 \pm 0.002	0.15 \pm 0.001
Throat hue	34.14	3	<0.001	0.96 \pm 0.02	0.92 \pm 0.02	1.06 \pm 0.02	1.07 \pm 0.02
Back brightness	0.70	3	0.871	31.93 \pm 3.23	32.94 \pm 2.12	32.88 \pm 2.55	35.94 \pm 2.33
Back saturation	3.94	3	0.268	0.13 \pm 0.006	0.14 \pm 0.004	0.12 \pm 0.005	0.13 \pm 0.004
Back hue	4.59	3	0.204	0.65 \pm 0.01	0.61 \pm 0.009	0.62 \pm 0.02	0.60 \pm 0.01
Belly brightness	2.96	3	0.396	198.42 \pm 12.47	175.39 \pm 9.50	188.67 \pm 10.80	185.08 \pm 7.78
Belly saturation	7.43	3	0.059	0.14 \pm 0.003	0.14 \pm 0.002	0.14 \pm 0.003	0.14 \pm 0.002
Belly hue	2.02	3	0.567	1.04 \pm 0.02	0.97 \pm 0.03	1.01 \pm 0.02	0.96 \pm 0.04

morph females (pairwise Wilcoxon rank-sum test: $P = 0.006$), more saturated (more pigmented) lateral crown stripe coloration than tan morph females and males ($P < 0.001$ and 0.002 , respectively), and yellower, more saturated superciliaries than tan morph birds of both sexes ($P < 0.001$).

Further, many plumage coloration variables also differed as a function of morph, across both sexes. White morph birds (both males and females) had brighter yellow, carotenoid-based superciliary stripes than tan morph birds (both males and females; pairwise Wilcoxon rank-sum tests: $P < 0.005$ in all cases). However, males and females did not differ in superciliary brightness within morphs ($P > 0.50$). Superciliary hue also differed only between the morphs ($P < 0.01$ for between-morph comparisons; $P > 0.50$ for within-morph comparisons), with white morph birds having greater hue than tan morph birds. Finally, differences in throat coloration were also between the

morphs, rather than between the sexes within the morphs. White morph males had brighter throats than both tan females ($P < 0.001$) and males ($P < 0.001$), and white morph females had brighter throats than tan males ($P = 0.01$) and tended to have brighter throats than tan females ($P = 0.06$). White morph birds (both males and females) also had greater throat hue than tan birds (both males and females; $P < 0.02$ for all comparisons).

Discriminant Function Analysis: Classifying Birds on the Basis of Coloration

Median crown stripe brightness, median crown stripe hue, lateral crown stripe brightness, lateral crown stripe saturation, superciliary brightness, superciliary hue, superciliary (carotenoid) saturation, throat brightness, and throat hue differed significantly between classes of birds. Thus, we used these variables in our discriminant function analysis (DFA). The DFA extracted 3 discriminant functions, the first 2 of which showed highly significant correlations to group membership (Table 2). The first discriminant function (DF1) described 78.4% of color variation between groups and was positively correlated with median crown stripe brightness, median crown stripe hue, superciliary brightness, superciliary hue, throat brightness, throat hue, and superciliary saturation. In addition, DF1 was also somewhat positively correlated with lateral crown stripe saturation, and negatively correlated with lateral crown stripe brightness (Table 3A). Thus, birds with higher scores on DF1 had brighter white and yellow coloration, more saturated (yellower) superciliaries, and somewhat darker (less bright) lateral crown stripes. The second discriminant function (DF2) described an addi-

TABLE 2. Variation described and significance tests for linear discriminant functions.

Function	Eigenvalue	Percentage of variance	Cumulative percentage	Canonical correlation
1	1.421	78.4	78.4	0.766
2	0.264	14.6	93.0	0.457
3	0.128	7.0	100.0	0.337
	Wilks's lambda	Chi-square	df	<i>P</i>
1–3	0.290	115.822	27	<0.001
2–3	0.701	33.158	16	0.007
3	0.887	11.242	7	0.128

TABLE 3. (A) Pooled within-group correlations (loadings) between discriminating variables and standardized canonical discriminant functions (DF1, DF2, DF3). (B) Values of functions at group centroids for each class of White-throated Sparrows, with significance tests for group differences. Abbreviations: TF = tan female, TM = tan male, WF = white female, and WM = white male, MCS = median crown stripe, and LCS = lateral crown stripe.

(A)			
Variable	DF1	DF2	DF3
MCS brightness	0.705*	-0.265	0.563
Superciliary brightness	0.654*	0.277	-0.286
MCS hue	0.645*	0.000	0.571
Superciliary hue	0.585*	0.456	-0.236
Throat hue	0.549*	0.523	0.172
Throat brightness	0.540*	0.355	0.136
Superciliary saturation	0.454*	-0.019	-0.146
LCS brightness	-0.292	0.526*	0.422
LCS saturation	0.416	-0.439*	0.053

(B)											
	Function			<i>P</i> (DF1) ^a				<i>P</i> (DF2) ^b			
	DF1	DF2	DF3	WM	WF	TM	TF	WM	WF	TM	TF
WM	1.288	-0.125	0.168	-	0.01	<0.001	<0.001	-	0.001	0.45	0.45
WF	0.348	0.895	-0.604	0.01	-	<0.001	<0.001	0.001	-	<0.001	0.45
TM	-1.038	-0.535	-0.234	<0.001	<0.001	-	0.45	0.45	<0.001	-	0.049
TF	-1.484	0.543	0.515	<0.001	<0.001	0.45	-	0.45	0.45	0.049	-

^a*P* values from Tukey's HSD tests.

^b*P* values from pairwise Wilcoxon rank-sum tests with Holm correction.

tional 14.6% of color variation and was positively correlated with lateral crown stripe brightness but negatively correlated with lateral crown stripe saturation. Thus, birds with higher scores on DF2 had less pigmented and brighter (less black) lateral crown stripe coloration (Table 3A). DF1 appeared to distinguish the morphs, and also the sexes within the white morph, with white morph females having DF1 scores intermediate between white morph males and tan morph birds (Figure 3). By contrast, tan males and females did not appear to differ with respect to DF1 (Figure 3). DF2 appeared to distinguish males from females, with females showing higher scores (less saturated and brighter lateral crown stripes) than males (Figure 3). Tukey's HSD and paired Wilcoxon rank-sum post hoc tests assessing differences in DF1 and DF2 scores, respectively, confirmed the above conclusions (Table 3B).

With prior probabilities calculated from the dataset, the DFA correctly classified 69.3% of birds into the appropriate morph–sex class. In this case, classification success was higher for males than for females, because DFA is particularly likely to misclassify the members of smaller groups when prior probabilities are calculated in this way, and fewer females were sampled than males (Table 4A). With the exception of tan males, most birds were misclassified as the opposite sex of the same morph. However, tan morph males were misclassified as white morph males at the highest rate (Table 4A). With prior probabilities set equal at 25% for all morph–sex classes,

success of classification was 65.3%, and the discrepancy in classification errors between males and females was eliminated (Table 4A). Again, most birds were misclassified as the opposite sex of the same morph. The highest rate of misclassification was for tan morph females, which were classified 35.3% of the time as tan males (Table 4B). In both cases, classification by DFA led to a >30% increase

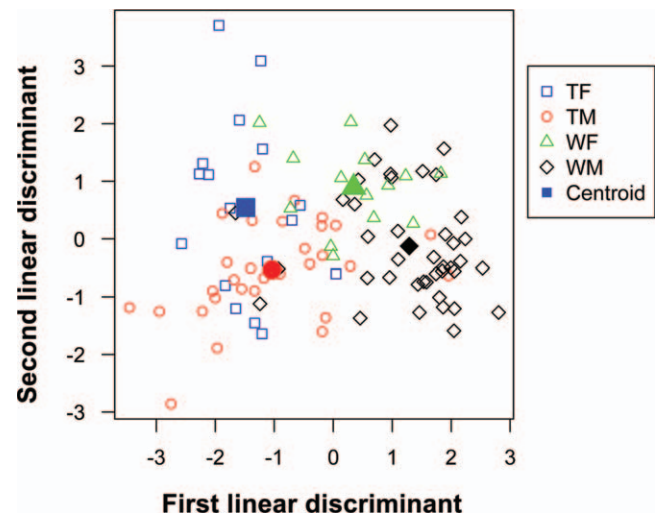


FIGURE 3. Plot of individuals and group centroids on the first and second linear discriminant functions extracted from plumage coloration variables. Abbreviations: TF = tan female, TM = tan male, WF = white female, and WM = white male.

TABLE 4. Classification of birds into morph and sex classes by discriminant function analysis. (A) Prior probabilities calculated from group membership (69.3% of 101 birds classified correctly). (B) Prior probabilities set equal at 25% each (66.3% of 101 birds classified correctly). Abbreviations: WM = white morph male, WF = white morph female, TM = tan morph male, and TF = tan morph female.

		Predicted group membership					
		Type	WM	WF	TM	TF	Total
Count	WM		31	6	3	0	42
	WF		4	7	2	1	14
	TM		4	1	23	2	28
	TF		0	0	8	9	17
Percentage	WM		77.5	15.0	7.5	0	100.0
	WF		28.6	50.0	14.3	7.1	100.0
	TM		13.3	3.3	76.7	6.7	100.0
	TF		0	0	47.1	52.9	100.0

		Predicted group membership					
		Type	WM	WF	TM	TF	Total
Count	WM		27	10	2	1	40
	WF		2	11	0	1	14
	TM		3	3	19	5	30
	TF		0	1	6	10	17
Percentage	WM		67.5	25.0	5.0	2.5	100.0
	WF		14.3	78.6	0	7.1	100.0
	TM		10.0	10.0	63.3	16.7	100.0
	TF		0	5.9	35.3	58.8	100.0

in classification success compared with classification by chance alone, in which case 29.2% of birds are expected to be classified correctly (by classifying birds into groups based on prior probabilities calculated from the dataset). Chi-square tests indicated that these increases in classification success were statistically significant ($\chi^2_1 = 78.58$, $P < 0.001$; $\chi^2_1 = 67.37$, $P < 0.001$).

Variation in plumage traits. Variation in plumage coloration existed within each morph–sex class (Figure 3). Variance in the coloration of body feather patches did not differ between the classes (Levene's tests: $P > 0.30$). However, differences in variance did emerge in the case of crown feather patches. White morph males showed more variance in the brightness of the median crown stripe than both tan morph males ($F_{1,68} = 10.17$, $P = 0.002$) and females ($F_{1,55} = 11.12$, $P = 0.002$), and more variance in median crown stripe hue than tan morph females ($F_{1,55} = 6.08$, $P = 0.02$). Further, white morph males were more variable in the hue of the lateral crown stripe than tan morph males ($F_{1,69} = 4.98$, $P = 0.03$) and white morph females ($F_{1,53} = 5.46$, $P = 0.02$). However, tan morph males showed higher variance in superciliary brightness than white males ($F_{1,69} = 7.35$, $P = 0.008$), and higher variance in

throat brightness than both white males ($F_{1,69} = 7.62$, $P = 0.007$) and white females ($F_{1,43} = 5.95$, $P = 0.02$). Finally, tan morph females showed the highest variance in lateral crown stripe brightness, having significantly higher variance than both tan males ($F_{1,45} = 5.88$, $P = 0.02$) and white males ($F_{1,56} = 6.43$, $P = 0.01$).

DISCUSSION

Different dimensions of variation in crown and throat plumage coloration distinguished the two morphs of the White-throated Sparrow, versus the sexes within the morphs, which suggests that crown coloration is a multifaceted signal. In contrast to head plumage coloration, body feathers showed no differences in coloration between classes of birds, which suggests that head plumage coloration is uniquely selected to serve a signaling function. Indeed, at the pre-alternate molt preceding the breeding season, White-throated Sparrows molt only their head feathers (Law 1929), which suggests that this signaling function may be particularly relevant to interactions on the breeding ground. Not surprisingly, in our discriminant function analysis, the first discriminant function distinguished white morph birds from tan morph birds. White morph birds were particularly characterized by greater median crown stripe brightness and hue, but also had brighter throats and superciliaries, and less bright (darker), more saturated lateral crown stripe coloration than tan morph birds. In addition to discriminating the morphs, the first discriminant function also distinguished white morph males from white morph females. Specifically, with respect to DF1, white morph females had coloration intermediate between tan morph birds and white morph males. However, within both morphs, the sexes were also differentiated along the second discriminant function, with females having less saturated and brighter lateral crown stripe coloration than males. These results suggest that median crown stripe coloration is particularly important in signaling morph identity. Indeed, initial morphing criteria in the White-throated Sparrow were based on coloration of the median crown stripe (Thornycroft 1966, 1975). By contrast, variation in melanin-based lateral crown stripe coloration appears to differentiate the sexes within the morphs. Given the multiple dimensions of variation in head plumage coloration, our analysis suggests that White-throated Sparrows should be able to distinguish between both sex and morph classes $\geq 66\%$ of the time and adopt appropriate behaviors accordingly.

The need to signal both morph and sex identity could have promoted the evolution of multifaceted plumage coloration in the White-throated Sparrow, as suggested by the multiple-messages hypothesis for the evolution of multiple ornaments (Møller and Pomiankowski 1993,

Johnstone 1995, Hebets and Papaj 2005). Specifically, expression of white median crown stripe coloration might be favored to signal morph identity, whereas expression of melanin-based lateral crown stripe coloration might be favored to signal sex identity. However, overall, results do not suggest that the signaling functions of different coloration patches are completely distinct, particularly because the coloration of multiple feather patches, including the median crown stripe, carotenoid-based superciliary stripe, and throat, differed between the 2 morphs. Thus, multiple color patches encode redundant (overlapping) information regarding morph, and birds may be able to more accurately distinguish between the morphs than if only one of these color patches were expressed. Therefore, results suggest that complex ornaments, and multifaceted color patterns in particular, may simultaneously convey multiple messages and promote more reliable signaling by providing redundant information (Møller and Pomiankowski 1993). Indeed, recent studies suggest that the multiple-messages and redundant-messages hypotheses are likely to represent complementary, rather than alternative, explanations for the evolution of multifaceted ornamental traits (Scheuber et al. 2004, Freeman-Gallant et al. 2010, Guindre-Parker et al. 2013).

As predicted, given stronger sexual selection for conspicuous plumage in white morph males than in tan morph males, more sexual dichromatism was evident within the white morph than within the tan morph. As described above, white morph males and females varied with respect to coloration along both the first and second discriminant functions, whereas tan morph males and females differed only with respect to the second discriminant function (which accounts for a lesser percentage of total variation in coloration than the first function: 14.6% vs. 78.4%). Further, in pairwise comparisons, white morph males and females differed significantly in the brightness and hue of the median crown stripe, whereas no significant differences in the coloration of crown patches occurred between tan morph males and females. These results suggest that sexual selection on white morph males has increased the conspicuousness of plumage coloration in these males. The difference in reproductive strategy between tan and white morph males, with white morph males being more highly aggressive and more promiscuous (Tuttle 2003), may lead to greater sexual selection on white morph males, and higher levels of sexual dichromatism within the white morph. Indeed, variance in reproductive success is higher within the white morph (E. M. Tuttle personal observation), which is consistent with stronger sexual selection acting on white morph males. In other species, greater promiscuity and higher rates of extrapair paternity have been associated with higher levels of sexual dimorphism and dichromatism (Dunn et al. 2001, Badyaev and Hill 2003). In addition, natural selection on white

morph females may have acted to reduce plumage conspicuousness in comparison to white morph males, given that duller pigmentation may lower nest predation and incidences of misdirected aggression (Martin and Badyaev 1996, Badyaev and Hill 2003, Coady and Dawson 2013). Our study is consistent with the hypothesis that differences in sexual and natural selection related to reproductive strategies underlie the evolution of sexual dichromatism, in that the 2 morphs share a common environment and genome apart from the inverted portion of the second chromosome, but differ in reproductive strategy (Tuttle 2003).

Despite differences in coloration between the sexes, birds were most frequently misclassified as the opposite sex of the same morph, even within the white morph, which suggests that plumage characteristics more strongly differentiate the morphs than the sexes. In addition to plumage coloration, behavioral cues such as song, solicitation behavior, and aggressive displays may serve to distinguish the sexes. Strong plumage differences between morphs may have evolved to facilitate adaptive social pairing patterns, since pairing is almost exclusively disassortative in the White-throated Sparrow (Lowther 1961, Houtman and Falls 1994, Tuttle 2003). Distinct crown plumage characteristics may prevent white morph birds from pairing together and producing offspring homozygous for the inverted region of the second chromosome, which is hypothesized to depress fitness, given its rare occurrence in the population (Thornycroft 1975, Tuttle 2003, Romanov et al. 2009). Similarly, in other polymorphic species, distinctive plumage characteristics also serve to prevent maladaptive pairing patterns. For instance, Gouldian Finches pair assortatively by color morph, with disassortative mating being associated with production of inviable offspring (Pryke and Griffith 2009a, 2009b, Pryke 2010). Other species, such as the Parasitic Jaeger (*Stercorarius parasiticus*), the Lesser Snow Goose (*Chen caerulescens caerulescens*), and the Bananaquit (*Coereba flaveola*) also have genetic color polymorphisms and may exhibit morph-specific mating patterns (Cooke et al. 1976, 1995, Wunderle 1981a, 1981b, O'Donald 1983, Phillips and Furness 1998). Furthermore, distinctive color patterns in the two morphs of the White-throated Sparrow also likely serve to signal differences in aggression, given that both males and females of the white morph are more highly aggressive than tan morph birds (Kopachena and Falls 1993a, Tuttle 2003). One caveat, however: Because our study is correlative rather than experimental, we cannot conclusively determine the extent to which White-throated Sparrows use coloration versus behavioral cues to distinguish classes of birds.

The genetic basis for differences in coloration between morphs of the White-throated Sparrow remains to be established. However, in other species with genetically

based color morphs, differences in coloration appear to be genetically dictated by mutations to the melanocortin-1 receptor (MC1R) gene, which controls melanin deposition in feathers (reviewed in Mundy 2005, Hubbard et al. 2010). Thus, this gene may be responsible for differences in coloration between the 2 morphs. Specifically, distinct MC1R alleles in tan morph birds may cause more melanin to be produced, leading to the darkening of white median crown stripe feathers and yellow superciliaries that is characteristic of this morph (Thornycroft 1975, N. A. Rathbun personal observation). In addition, the fact that male and female birds differed in coloration within a morph suggests that both morph- and sex-specific genetic effects on pigmentation exist, and that selection can differentiate male and female plumage coloration within morphs, despite coinheritance of unique alleles associated with the inverted region of the second chromosome.

Finally, the variation observed in plumage coloration within each morph–sex class indicates that there is a basis for selection to act on plumage coloration, and for coloration to signal individual quality differences (Andersson 1994). The brightness and hue of the median crown stripe were more variable in white males than in other classes of birds. A bright white median crown stripe, which is associated with white male identity, might also serve as an effective signal of individual quality in white morph males if signaling white male identity has social costs. Indeed, white morph males are more aggressive toward same-morph territorial intruders (Horton et al. 2012, Laubach et al. 2013). By contrast, contrary to our prediction that carotenoid-based pigmentation would be highly variable in white morph males compared with other classes of birds, carotenoid-based superciliary coloration and white throat coloration were more highly variable in tan morph males, which might suggest that these feather patches have more potential to signal individual quality differences in tan morph males. However, determining the relationship between individual quality and variation in coloration will require further research.

In summary, our results demonstrate that different axes of variation in crown plumage coloration distinguish the morph and sex classes in the White-throated Sparrow, which suggests that crown plumage coloration is a multifaceted signal and that individuals should be able to distinguish among classes of birds on the basis of crown coloration. Greater sexual dichromatism in white morph birds than in tan morph birds supports a role for sexual selection in the evolution of sexual dichromatism, given that white morph males are predicted to be under stronger sexual selection for conspicuous coloration than tan morph birds (Tuttle 2003). Further, differences in which crown coloration traits were most variable within the 2 morphs of males suggest that different crown coloration characteristics may be most useful as sexual signals within

the 2 morphs. The next step in elucidating the evolution of plumage signals in the White-throated Sparrow will involve determining whether plumage coloration is differentially correlated with quality and fitness metrics in the 2 morphs and sexes. Overall, our results suggest that multifaceted plumage coloration can simultaneously play both multiple and redundant messaging functions, and that complex coloration in polymorphic species may be selected to convey multiple messages regarding morph, sex, and associated behavioral strategies.

ACKNOWLEDGMENTS

We acknowledge the 2006–2009 White-throated Sparrow field crews; Cranberry Lake Biological Station; The Center for Genomic Advocacy at Indiana State University; and M. Korody, P. Sebastian, A. Jamison, M. Baird, C. A. T. Gonser, and Zonotrichia Zeke. Funding sources included the School of Graduate and Professional Studies at Indiana State University; Sigma-Xi Grants-in-Aid-of-Research (to N.A.R.); University of Colorado; and National Science Foundation grant DUE-0934648 and National Institutes of Health grant 1R01GM084229 (to E.M.T. and R.A.G.). All research reported here was in compliance with the current laws of New York State, the State of Indiana, and the U.S. federal government.

LITERATURE CITED

- Alonso-Alvarez, C., L. Pérez-Rodríguez, R. Mateo, O. Chastel, and J. Viñuela (2008). The oxidative handicap hypothesis and the carotenoid allocation trade-off. *Journal of Evolutionary Biology* 21:1769–1797.
- Andersson, M. (1994). *Sexual Selection*. Princeton University Press, Princeton, NJ, USA.
- Andersson, S., and M. Prager (2006). Quantifying colors. In *Bird Coloration*, vol. 1: Mechanisms and Measurements (G. E. Hill and K. J. McGraw, Editors). Harvard University Press, Cambridge, MA, USA.
- Badyaev, A. V., and G. E. Hill (2003). Avian sexual dichromatism in relation to phylogeny and ecology. *Annual Reviews in Ecology and Evolutionary Systems* 34:27–49.
- Britton, G., S. Liaaen-Jensen, and H. Pfander (Editors) (1995). *Carotenoids*, vol. 1A: Isolation and Analysis. Birkhäuser, Basel, Switzerland.
- Brommer, J. E., K. Ahola, and T. Karstinen (2005). The colour of fitness: Plumage coloration and lifetime reproductive success in the tawny owl. *Proceedings of the Royal Society of London, Series B* 272:935–940.
- Burns, K. J., and A. J. Shultz (2012). Widespread cryptic dichromatism and ultraviolet reflectance in the largest radiation of Neotropical songbirds: Implications of accounting for avian vision in the study of plumage evolution. *The Auk* 129:211–221.
- Coady, C. D., and R. D. Dawson (2013). Subadult plumage color of female Tree Swallows (*Tachycineta bicolor*) reduces conspecific aggression during the breeding season. *Wilson Journal of Ornithology* 125:348–357.

- Cooke, F., G. Finney, and R. Rockwell (1976). Assortative mating in Lesser Snow Geese (*Anser caerulescens*). *Behavior Genetics* 6:127–140.
- Cooke, F., R. F. Rockwell, and D. B. Lank (1995). The Snow Geese of La Pérouse Bay. Oxford University Press, Oxford, UK.
- Cuthill, I. C., A. T. D. Bennett, J. C. Partridge, and E. H. Maier (1999). Plumage reflectance and the objective assessment of avian sexual dichromatism. *American Naturalist* 160:183–200.
- Dale, J. (2000). Ornamental plumage does not signal male quality in Red-billed Queleas. *Proceedings of the Royal Society of London, Series B* 267:2143–2149.
- Darwin, C. (1871). *The Descent of Man and Selection in Relation to Sex*. John Murray, London, UK.
- Doucet, S. M., D. J. Mennill, R. Montgomerie, P. T. Boag, and L. M. Ratcliffe (2005). Achromatic plumage reflectance predicts reproductive success in male Black-capped Chickadees. *Behavioral Ecology* 16:218–222.
- Dunn, P. O., L. A. Whittingham, and T. E. Pitcher (2001). Mating systems, sperm competition, and the evolution of sexual dimorphism in birds. *Evolution* 55:161–175.
- Ellegren, H. (1996). First gene on the avian W chromosome (CHD) provided a tag for universal sexing of non-ratite birds. *Proceedings of the Royal Society of London, Series B* 263:1635–1641.
- Formica, V. A., R. A. Gonser, S. M. Ramsay, and E. M. Tuttle (2004). Spatial dynamics of alternative reproductive strategies: The role of neighbors. *Ecology* 85:1125–1136.
- Formica, V. A., and E. M. Tuttle (2009). Examining the social landscapes of alternative reproductive strategies. *Journal of Evolutionary Biology* 22:2395–2408.
- Freeman-Gallant, C. R., C. C. Taff, D. F. Morin, P. O. Dunn, L. A. Whittingham, and S. M. Tsang (2010). Sexual selection, multiple male ornaments, and age- and condition-dependent signaling in the Common Yellowthroat. *Evolution* 64:1000–1017.
- Griffiths, R., M. C. Double, K. Orr, and R. J. G. Dawson (1998). A DNA test to sex most birds. *Molecular Ecology* 7:1071–1075.
- Grunst, A. S., J. T. Rotenberry, and M. L. Grunst (2014). Age-dependent relationships between multiple sexual pigments and condition in males and females. *Behavioral Ecology* 25:276–287.
- Guindre-Parker, S., H. G. Gilchrist, S. Baldo, S. M. Doucet, and O. P. Love (2013). Multiple achromatic plumage ornaments signal to multiple receivers. *Behavioral Ecology* 24:672–682.
- Hebets, E. A., and D. R. Papaj (2005). Complex signal function: Developing a framework of testable hypotheses. *Behavioral Ecology and Sociobiology* 57:197–214.
- Hegyi, G., B. Szigeti, J. Török, and M. Eens (2007). Melanin, carotenoid, and structural plumage ornaments: Information content and role in Great Tits *Parus major*. *Journal of Avian Biology* 38:698–708.
- Hill, G. E. (1991). Plumage coloration is a sexually selected indicator of male quality. *Nature* 350:337–339.
- Hill, G. E. (2002). *A Red Bird in a Brown Bag: The Function and Evolution of Colorful Plumage in the House Finch*. Oxford University Press, New York, NY, USA.
- Holm, S. (1979). A simple sequentially rejective multiple test procedure. *Scandinavian Journal of Statistics* 6:65–70.
- Hórák, P., I. Ots, H. Vellau, C. Spottiswoode, and A. P. Møller (2001). Carotenoid-based plumage coloration reflects hemoparasite infection and local survival in breeding Great Tits. *Oecologia* 126:166–173.
- Horton, B. M., M. E. Hauber, and D. L. Maney (2012). Morph matters: Aggression bias in a polymorphic sparrow. *PLoS ONE* 7:e48705.
- Houtman, A., and J. B. Falls (1994). Negative assortative mating in the White-throated Sparrow, *Zonotrichia albicollis*: The role of mate choice and intra-sexual competition. *Animal Behaviour* 48:377–383.
- Hubbard, J. K., J. A. C. Uy, M. E. Hauber, H. E. Hoekstra, and R. J. Safran (2010). Vertebrate pigmentation: From underlying genes to adaptive function. *Trends in Genetics* 26:231–239.
- Johnstone, R. A. (1995). Honest advertisement of multiple qualities using multiple signals. *Journal of Theoretical Biology* 177:87–94.
- Jukema, J., and T. Piersma (2006). Permanent female mimics in a lekking shorebird. *Biology Letters* 2:161–164.
- Keyser, A., and G. E. Hill (1999). Condition-dependent variation in the blue-ultraviolet coloration of a structurally based plumage ornament. *Proceedings of the Royal Society of London, Series B* 266:771–778.
- Knapton, R. W., and J. B. Falls (1983). Differences in parental contribution among pair types in the polymorphic White-throated Sparrow. *Canadian Journal of Zoology* 61:1288–1292.
- Kopachena, J. G., and J. B. Falls (1991). An experimental study of brood division in White-throated Sparrows. *Animal Behaviour* 42:395–402.
- Kopachena, J. G., and J. B. Falls (1993a). Aggressive performance as a behavioral correlate of plumage polymorphism in the White-throated Sparrow (*Zonotrichia albicollis*). *Behaviour* 124:249–266.
- Kopachena, J. G., and J. B. Falls (1993b). Postfledging parental care in the White-throated Sparrow (*Zonotrichia albicollis*). *Canadian Journal of Zoology* 71:227–232.
- Korody, M. L. (2006). Factors influencing sex ratio variation within the polymorphic White-throated Sparrow. M.S. thesis, Indiana State University, Terre Haute, IN, USA.
- Lank, D. B., C. M. Smith, O. Hanotte, T. Burke, and F. Cooke (1995). Genetic polymorphism for alternative mating behaviour in lekking male Ruff (*Philomachus pugnax*). *Nature* 378:59–62.
- Laubach, Z. M., D. T. Blumstein, L. M. Romero, G. Sampson, and J. Foufopoulos (2013). Are White-crowned Sparrow badges reliable signals? *Behavioral Ecology and Sociobiology* 67:481–492.
- Law, J. E. (1929). The spring molt in *Zonotrichia*. *The Condor* 31:208–212.
- Le Corre, M. (1999). Plumage Polymorphism of red-footed boobies in the western Indian Ocean: An indicator of biogeographic isolation. *Journal of Zoology* 249:411–415.
- Longmire, J. L., G. F. Gee, O. L. Handenkipf, and G. A. Mark (1992). Establishing paternity in Whooping Cranes, *Gus americana*, by DNA analysis. *The Auk* 109:522–529.
- Lowther, J. K. (1961). Polymorphism in the White-throated Sparrow, *Zonotrichia albicollis* (Gmelin). *Canadian Journal of Zoology* 39:281–292.
- Martin, T. E., and A. V. Badyaev (1996). Sexual dichromatism in birds: Importance of nest predation and nest location for females versus males. *Evolution* 50:2454–2460.

- McGraw, K. J. (2003). Melanins, metals, and mate quality. *Oikos* 102:402–406.
- McGraw, K. J. (2006). Mechanics of carotenoid coloration. In *Bird Coloration*, vol. 1: Measurements and Mechanisms (G. E. Hill and K. J. McGraw, Editors). Harvard University Press, Cambridge, MA, USA.
- McGraw, K. J., R. J. Safran, and K. Wakamatsu (2005). How feather colour reflects its melanin content. *Functional Ecology* 19: 816–821.
- Michopoulos, V., D. L. Maney, C. B. Morehouse, and J. W. Thomas (2007). A genotyping assay to determine plumage morph in the White-throated Sparrow (*Zonotrichia albicollis*). *The Auk* 124:1330–1335.
- Møller, A. P., and A. Pomiankowski (1993). Why have birds got multiple sexual ornaments? *Behavioral Ecology and Sociobiology* 32:167–176.
- Montgomerie, R. (2008). CLR, version 1.05. Queen's University, Kingston, Canada. <http://post.queensu.ca/~mont/color/analyze.html>
- Mundy, N. I. (2005). A window on the genetics of evolution: MC1R and plumage colouration in birds. *Proceedings of the Royal Society of London, Series B* 272:1633–1640.
- O'Donald, P. (1983). *The Arctic Skua: A Study of the Ecology and Evolution of a Seabird*. Cambridge University Press, Cambridge, UK.
- Phillips, R. A., and R. W. Furness (1998). Polymorphism, mating preferences and sexual selection in the Arctic Skua. *Journal of Zoology* 245:245–252.
- Piper, W. H., and R. H. Wiley (1989). Distinguishing morphs of the White-throated Sparrow in basic plumage. *Journal of Field Ornithology* 60:73–83.
- Pryke, S. R. (2010). Sex chromosome linkage of mate preference and color signal maintains assortative mating between interbreeding finch morphs. *Evolution* 64:1301–1310.
- Pryke, S. R., and S. C. Griffith (2006). Red dominates black: Agonistic signalling among head morphs in the colour polymorphic Gouldian Finch. *Proceedings of the Royal Society of London, Series B* 273:949–957.
- Pryke, S. R., and S. C. Griffith (2007). The relative role of male vs. female mate choice in maintaining assortative pairing among discrete colour morphs. *Journal of Evolutionary Biology* 20: 1512–1521.
- Pryke, S. R., and S. C. Griffith (2009a). Genetic incompatibility drives sex allocation and maternal investment in a polymorphic finch. *Science* 323:1605–1607.
- Pryke, S. R., and S. C. Griffith (2009b). Postzygotic genetic incompatibility between sympatric color morphs. *Evolution* 63:793–798.
- R Core Team (2012). R: A Language and Environment for Statistical Computing. R Foundation for Statistical Computing, Vienna Austria). <http://www.R-project.org/>
- Rohwer, S. A. (1975). The social significance of avian winter plumage variability. *Evolution* 29:593–610.
- Romanov, M. N., E. M. Tuttle, M. L. Houck, W. S. Modi, L. G. Chemnick, M. L. Korody, E. M. S. Mork, C. Otten, T. Renner, K. C. Jones, S. Dandekar, J. C. Papp, et al. (2009). The value of avian genomics to the conservation of wildlife. *BMC Genomics* 10 (Supplement 2):S10.
- Roulin, A., P. Bize, P. A. Ravussin, and L. Broch (2004). Genetic and environmental effects on the covariation between colour polymorphism and a life history trait. *Evolutionary Ecology Research* 6:1253–1260.
- Safran, R. J., and K. J. McGraw (2004). Plumage coloration, not length or symmetry of tail-streamers, is a sexually selected trait in North American Barn Swallows. *Behavioral Ecology* 15: 455–461.
- Scheuber, H., A. Jacot, and M. W. G. Brinkhof (2004). Female preference for multiple condition-dependent components of a sexually selected signal. *Proceedings of the Royal Society of London, Series B* 271:2453–2457.
- Siefferman, L., and G. E. Hill (2005). UV-blue structural coloration and competition for nestboxes in male Eastern Bluebirds. *Animal Behaviour* 69:67–72.
- Thomas, J. W., M. Cáceres, J. J. Lowman, C. B. Morehouse, M. E. Short, E. L. Baldwin, D. L. Maney, and C. L. Martin (2008). The chromosomal polymorphism linked to variation in social behavior in the White-throated Sparrow (*Zonotrichia albicollis*) is a complex rearrangement and suppressor of recombination. *Genetics* 179:1455–1468.
- Thornycroft, H. B. (1966). Chromosomal polymorphism in the White-throated Sparrow, *Zonotrichia albicollis* (Gmelin). *Science* 154:1571–1572.
- Thornycroft, H. B. (1975). A cytogenetic study of the White-throated Sparrow, *Zonotrichia albicollis* (Gmelin). *Evolution* 29:611–621.
- Tuttle, E. M. (1993). Mate choice and the maintenance of stable polymorphisms in the White-throated Sparrow. Ph.D. dissertation, State University of New York at Albany, Albany, NY, USA.
- Tuttle, E. M. (2003). Alternative reproductive strategies in the White-throated Sparrow: Behavioral and genetic evidence. *Behavioral Ecology* 14:425–432.
- Tuttle, E. M., R. R. Jensen, V. A. Formica, et al. (2006). Using remote sensing image texture to study habitat use patterns: A case study using the polymorphic white-throated sparrow (*Zonotrichia albicollis*). *Global Ecology and Biogeography* 15: 349–357.
- Wunderle, J. M., Jr. (1981a). An analysis of a morph ratio cline in the Bananaquit (*Coereba flaveola*) on Grenada, West Indies. *Evolution* 35:333–344.
- Wunderle, J. M., Jr. (1981b). Colour phases of the Bananaquit *Coereba flaveola* on St Vincent, West Indies. *Ibis* 123:354–358.