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COLOR DIFFERENCES AMONG CLOSELY RELATED SPECIES OF RED-BREASTED MEADOWLARKS (*STURNELLA*)

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Abstract. Interspecific differences in sexually selected traits may be important for maintaining reproductive isolation among closely related species living in sympatry. We present the first study of plumage color differences among males of partially sympatric species of South American red-breasted meadowlarks—the White-browed Blackbird (*Sturnella superciliaris*), the Pampas Meadowlark (*S. defilippii*), and the Long-tailed Meadowlark (*S. loyca*)—using reflectance spectrophotometry and the avian visual model of Vorobyev and Osorio (1998). Reflectance values of sexually dichromatic red plumage patches were measured on study skins. Total reflectance, reflectance in the short wavelength part of the spectrum, and several measures of spectral shape were extracted directly from the spectra. Our analyses revealed that *S. loyca* and *S. defilippii* were brighter and had higher reflectance in the short wavelength part of the spectrum than *S. superciliaris*. Minimum reflectance was located at higher wavelengths in breeding than in nonbreeding plumage. Interspecific distances in avian visual space obtained from the Vorobyev and Osorio (1998) model were considerably higher than the threshold value for color discrimination, indicating that the differences found are also detectable by birds. Taken together, these results show that the red plumage patches of these three species present significant color differences throughout the year, not only in the visible but also in the UV part of the spectrum.

Key words: male plumage, meadowlarks, reflectance, spectrophotometry, Sturnella*, sympatry.*

Diferencias de Coloración entre Especies Cercanamente Emparentadas de Pechos Colorados (*Sturnella*)

Resumen. Las diferencias interespecíficas en caracteres sexualmente seleccionados pueden ser importantes para mantener el aislamiento reproductivo entre especies simpátricas cercanamente emparentadas. Este es el primer estudio de las diferencias de coloración de plumaje entre machos de especies parcialmente simpátricas de pechos colorados sudamericanos (*Sturnella superciliaris, S. defilippii*, y *S. loyca*) usando espectrofotometría de reflectancia y el modelo de percepción visual de Vorobyev y Osorio (1998). Los valores de reflectancia de los parches rojos sexualmente dimórficos fueron medidos en pieles de estudio. La reflectancia total, la reflectancia en la porción de onda corta del espectro y varias medidas de forma espectral fueron obtenidas directamente de los espectros. Nuestros análisis revelaron que *S. loyca* y *S. defilippii* fueron más brillantes y tuvieron mayor reflectancia en la región de onda corta del espectro que *S. superciliaris*. La posición de reflectancia mínima se localizó a longitudes de onda mayores en el plumaje reproductivo comparado con el no reproductivo. Las distancias interespecíficas en el espacio visual aviano obtenidas del modelo de Vorobyev y Osorio (1998) fueron considerablemente mayores que el valor umbral de discriminación de color, indicando que estas diferencias son también detectables por las aves. Tomados en su conjunto, estos resultados muestran que los parches de plumaje rojo de estas tres especies presentan diferencias significativas en la coloración durante todo el año tanto en la porción visible del espectro como en la UV.

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INTRODUCTION

A central endeavor in evolutionary biology is the investigation of mechanisms of reproductive isolation between closely related species. Theoretical arguments indicate that precopulatory mechanisms, such as visual signals employed in species recognition and sexual selection, should be among the most important reproductive barriers (Coyne and Orr 2004). In particular, differences in sexual signals among morphologically similar, closely related species should be greater in sympatry, where chances of confusion and hybridization are at a maximum compared to allopatry (Miller 1982).

The genus *Sturnella* is comprised of seven species living in open grasslands or open steppes of the Americas (Short 1968). *Sturnella* species fall into two broad categories with respect to breast coloration: the yellow-breasted meadowlarks (*Sturnella magna* [Eastern Meadowlark] and *S. neglecta* [Western Meadowlark]) and the red-breasted meadowlarks, comprised of the five remaining species (*S. militaris* [Red-breasted Blackbird], *S. superciliaris* [White-browed Blackbird], *S. bellicosa* [Peruvian Meadowlark], *S. defilppii* [Pampas Meadowlark], and *S. loyca* [Long-tailed Meadowlark]). In this genus there are several pairs or trios of morphologically similar (sibling) species: *S. magna* and *S. neglecta*; *S. bellicosa, S. defilippii*, and *S. loyca*; and *S. militaris* and *S. superciliaris*. Some of these species are sympatric over at least part of their ranges. This is true for three Argentine *Sturnella* species, *S. superciliaris, S. defilippii*, and *S. loyca*, which coexist in the southern pampas.

Red-breasted meadowlarks are sexually dimorphic in color, mainly in the extent and intensity of the red patches located on the throat, chest, belly, and marginal wing coverts (Gochfeld 1975). This, together with the use of a flight display by most males of the species in the red-breasted group, suggests that these plumage characters might be used as species recognition signals (Short 1968). If this is the case, however, these signals must be different among sympatric species.

Because of the striking differences in visual systems and color perception between humans and birds (Bennett and Cuthill 1994, Bowmaker et al. 1997, Cuthill et al. 2000a, 2000b), human description of plumage color is inadequate for the study of many biological questions. To overcome this problem, several studies have used reflectance spectrophotometry to assess differences among closely related species of birds, such as tanagers (Johnson and Brush 1972, Bleiweiss 2004a, 2005). Here we present the first quantitative and objective study of color differences among males of the three partially sympatric species of meadowlarks living in the Argentine pampas by means of reflectance spectrophotometry. In particular, we test for the existence of consistent differences in the red patches of plumage that might be used as species recognition signals. In addition, we examine seasonal changes in the red-colored patches and discuss this variation in relation to the pattern of molt and wear of the feathers. We present the results of the reflectance analysis alone and in combination with the avian visual perceptual model developed by Vorobyev and Osorio (1998). To our knowledge, this is the first study to use visual modeling methods to quantify differences in plumage color among bird species and its seasonal variation. Thus, plumage is assessed independently of human vision and differences in plumage color are evaluated in the way in which birds should perceive them.

METHODS

DATA COLLECTION

This study is based on study skins deposited at the Museo Argentino de Ciencias Naturales "Bernardino Rivadavia." Only specimens in perfect condition (without evidence of degradation due to poor storage conditions or defects in preparation) and with complete information on sex, locality, and date of collection were included, totaling 12 *S. superciliaris* (seven in breeding and five in nonbreeding plumage), 16 *S. defilippii* (10 in breeding and six in nonbreeding plumage), and 15 *S. loyca* (eight in breeding and seven in nonbreeding plumage) adult males. We considered birds collected between September and January to be in breeding plumage, and those collected between February and August to be in nonbreeding plumage. The collection dates of the individuals ranged from 1896 to 1979 (mean collection year $\pm$ standard deviation for *S. superciliaris* = 1931 $\pm$ 20, for *S. defilippii* = 1924 $\pm$ 13, and for *S. loyca* = 1940 $\pm$ 25). For each study skin, we measured four of the most conspicuous red-

colored patches: the throat, chest, belly, and marginal wing coverts (hereafter referred to as coverts). These regions were chosen for three reasons: 1) they are the areas that are more apparently dimorphic in color, suggesting that they could be involved in species recognition or sexual selection, 2) they appear homogeneously colored and thus are amenable to spectrophotometric analysis, and 3) they are of sufficient size to allow at least one reflectance measure. Three measurements were taken from the chest and belly, one at the center and one to each side of the midline, and the mean of these measurements was used in all analyses. The coverts of both wings and the throat were measured only once; the mean of the right and left wing was used in all analyses. Because some *S. defilippii* individuals were prepared with the head pointing ventrally, we were unable to obtain a throat measurement for eight individuals (seven in breeding and one in nonbreeding plumage).

REFLECTANCE SPECTROPHOTOMETRY

Plumage reflectance was measured using an Ocean Optics 2000 (Ocean Optics, Inc., Dunedin, Florida) spectrophotometer with a PX-2 pulsed xenon light source (effective range of emission from 220 to 750 nm) calibrated against a white standard of barium sulphate, following Osorio and Ham (2002) and Bleiweiss (2004a, 2004b, 2005). Before measuring each specimen, a new calibration was done to correct for possible shifts in the performance of the spectrophotometer. Plumage was illuminated and reflected light collected at 45° to the surface and from the proximal end of the feather. This procedure was adopted to avoid the effect of specular reflectance that can result when the feather is illuminated and reflected light collected perpendicular to its surface (Andersson 1996). The probe was mounted in a prismatic probe holder that was held (not pressed) over the selected region of the study skin. The diameter of the circular region illuminated and measured was approximately 6 mm, and the distance between the probe and the plumage was 23 mm. The spectrophotometer resolution was 0.35 nm. Each spectrum was the average of three readings with an integration time of 100 msec. Boxcar smoothing was not performed. Measurements were done blind to the season and year of capture of the specimens.

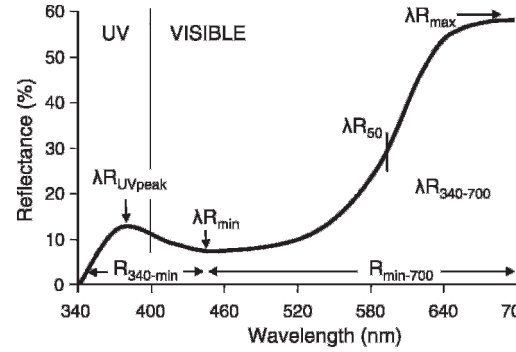

FIGURE 1. Representative reflectance spectrum of a red plumage patch of *Sturnella*, showing the different variables extracted from the spectral data. Variables include $R_{340–700}$ as the total reflectance from 340 to 700 nm, λR_{min} as the wavelength of the reflectance minimum, λR_{max} as the wavelength of the reflectance maximum, λR_{UVpeak} as the wavelength of the reflectance maximum in the near-UV part of the spectrum, and λR_{50}, calculated as the value midway between the wavelength of maximum and minimum reflectance ($\lambda R_{max} - \lambda R_{min}$), which accounts for the hue (spectral location of the reflectance band at visible wavelength). Two reflectance segments are determined by the wavelength of minimum reflectance (λR_{min}); one accounts for the reflectance between 340 nm and λR_{min} ($R_{340\text{-}min}$), and the other for the reflectance between λR_{min} and 700 nm ($R_{min\text{-}700}$). These two reflectance segments were used in the calculation of $R_{contrast}$ as $[(R_{min\text{-}700}) - (R_{340\text{-}min})]/(R_{340–700})$. Vertical line at 400 nm indicates the boundary between the near-UV wavelengths, from 340 to 400 nm (visible to birds), and the visible wavelengths, from 400 to 700 nm (visible to both humans and birds).

STATISTICAL ANALYSES

The original variables included in this study were median reflectance values for 5 nm bins from 340 to 700 nm for each individual. Reflectance values below 340 nm were excluded because of considerable noise at these wavelengths. For each spectrum we calculated eight variables (Fig. 1), following Pryke et al. (2001). Brightness ($R_{340–700}$, total reflectance) was calculated as the sum of the reflectance from 340 to 700 nm. We defined λR_{min} and λR_{max} as the wavelengths of minimum and maximum reflectance in the visible part of the spectrum, respectively. Hue (λR_{50}), the spectral location of the reflectance band at long visible wavelengths, was calculated as the value midway between that of maximum (λR_{max}) and minimum reflectance (λR_{min}). Because carotenoid pigmentation can exhibit a bimodal peak of

reflectance (Bleiweiss 2004b, 2005), we also measured the wavelength of maximum reflectance between 340 nm and λR_{min}. This variable was called λR_{UVpeak} and was the wavelength of maximum reflectance in the near-UV region of the spectrum. The reflectance at wavelengths lower than λR_{min} ($R_{340\text{-}min}$) and the reflectance at wavelengths higher than λR_{min} ($R_{min\text{-}700}$) were also measured and used to calculate reflectance contrast ($R_{contrast}$, a surrogate measure of chroma) as the relative difference between these two nonoverlapping spectral segments using the formula $[(R_{min\text{-}700}) - (R_{340\text{-}min})]/(R_{340\text{–}700})$. We used these variables in separate two-way ANCOVAs, with species and season (breeding versus nonbreeding) as main factors and year of capture as a covariate to control for possible effects of plumage fading with time since capture (Endler and Théry 1996, McNaught and Owens 2002). Although we measured eight variables, only five of them ($R_{340\text{–}700}$, λR_{min}, λR_{50}, λR_{UVpeak}, and $R_{contrast}$) were used in the ANCOVAs, because the other three (λR_{max}, $R_{340\text{-}min}$, and $R_{min\text{-}700}$) were only obtained to calculate other variables.

All statistical analyses were performed using Statistica version 6.0 (StatSoft 2001) and all tests were two-tailed as we made no a priori predictions as to which species would have higher values for any of the variables measured. ANCOVAs were followed by Scheffé contrasts for those variables that differed significantly among species. Because we made multiple comparisons using eight different variables for describing each body region, we used a Bonferroni-corrected $\alpha = 0.006$ to keep the global type I error smaller than 5%.

VISUAL MODELING

We evaluated color differentiation between *Sturnella* species for each feather patch, and between the breeding and nonbreeding seasons within each species, using the Vorobyev and Osorio (1998) color discrimination model. This model calculates distance in avian color space (ΔS), defined by the quantum catches of each receptor type in the avian retina. Thus, ΔS for a given feather patch represents the relative difference in color between species (or seasons) with respect to the avian visual system.

To calculate ΔS for each feather patch comparison, we first calculated the input to each avian cone cell type i of the average color among individuals for each feather patch, using the following equation (equation 1 in Vorobyev et al. 1998):

$$Q_i = \int_\lambda R_i(\lambda) S(\lambda) I(\lambda) d\lambda, \qquad (1)$$

where λ denotes wavelength, $R_i(\lambda)$ is the spectral sensitivity of cone cell type i (data taken from the Blue Tit [*Parus caeruleus*]; Hart et al. 2000), $S(\lambda)$ is the reflectance spectrum of a given feather patch, $I(\lambda)$ is the irradiance spectrum entering the eye, and integration is over 340–700 nm. Spectral sensitivity data, $R_i(\lambda)$, are not available for *Sturnella* species, therefore data from the Blue Tit were used as an approximation. These data likely provide a good estimate for other species, as passerine visual pigment characteristics (and thus cone cell sensitivities) are highly conserved over much of the visual range (Hart 2001). Because we were not studying the effects of viewing homologous feather patches in different light environments, we set $I(\lambda) = 1$ for all calculations.

The signal of each receptor type, f_i, is proportional to the natural logarithm of the respective quantum catch (Q_i from equation 1), which is normalized against an adapting background (see equation 2 and 3 in Vorobyev et al. [1998]). The distance (ΔS) between each homologous feather patch was then calculated using equation 8 in Vorobyev et al. (1998):

$$\begin{aligned}(\Delta S)^2 = \Big[&(\omega_1\omega_2)^2(\Delta f_4 - \Delta f_3)^2 \\ &+ (\omega_1\omega_3)^2(\Delta f_4 - \Delta f_2)^2 \\ &+ (\omega_1\omega_4)^2(\Delta f_3 - \Delta f_2)^2 \\ &+ (\omega_2\omega_3)^2(\Delta f_4 - \Delta f_1)^2 \\ &+ (\omega_2\omega_4)^2(\Delta f_3 - \Delta f_1)^2 \\ &+ (\omega_3\omega_4)^2(\Delta f_2 - \Delta f_1)^2\Big] \\ \div \Big[&(\omega_1\omega_2\omega_3)^2 \\ &+ (\omega_1\omega_2\,\omega_4)^2 \\ &+ (\omega_1\omega_3\,\omega_4)^2 \\ &+ (\omega_2\omega_3\,\omega_4)^2\Big], \qquad (2)\end{aligned}$$

where ω_i is the noise-to-signal ratio (Weber fraction) for receptor type i. We used Vorobyev

et al.'s (1998) estimate of the Weber fraction for the long-wave sensitive cone (LWS), $\omega_4 = 0.05$, which is based on an empirical estimate of behavioral data from the Pekin Robin (*Leiothrix lutea*). Relative values of ω_i are defined by the relative proportions of receptor types in the eye, thus the remaining ω_i were derived (see equation 10 in Vorobyev et al. [1998]) from estimates of the relative numbers of receptor cell types, η_i, based on anatomical data from the Blue Tit (Hart et al. 2000). Photoreceptor type proportions vary among closely related bird species, possibly in relation to differences in visual ecology (Hart 2001). Thus, we recalculated all ΔS with Weber fractions derived from cone cell proportions of European Starlings (*Sturnus vulgaris*) and European Blackbirds (*Turdus merula*), species with arguably different visual ecologies compared to the Blue Tit (Hart 2001). Although ΔS values did vary when calculated from the three different ratios of photoreceptor types of these three species, the magnitude of these differences changed none of our conclusions regarding interspecific and intraspecific color differentiation. Thus, all values reported and discussed are taken from the calculations based on the photoreceptor type ratios of the Blue Tit (see also Siddiqi et al. 2004, Eaton 2005).

The units of ΔS from equation 2 are jnd (just noticeable differences), where 1.0 jnd is the threshold value for discrimination of colors. Thus, ΔS values <1.0 jnd indicate that color differences are most likely not visually discernable to birds, while values >1.0 jnd indicate a magnitude of color difference above the visual discriminatory abilities of birds (Vorobyev et al. 1998, Vorobyev 2003, Siddiqi et al. 2004, Eaton 2005). Visual performance can vary among species and viewing conditions (Vorobyev et al. 1998), but, in general, at jnd = 1.0, two colors are barely distinguishable under ideal conditions, and as jnd increases two colors become more easily discernable under worsening viewing conditions (Siddiqi et al. 2004).

RESULTS

The shape of the reflectance curves for all three species showed a band of low reflectance in the range of 420–550 nm, with a minimum between 455 and 485 nm depending on the body region and species considered. This determined two segments of higher reflectance: a primary segment situated in the long wavelength part of the visible spectrum that accounted for most of the total reflectance, and a secondary and smaller peak in the near-UV and short wavelength portion of the visible spectrum (Fig. 2). Maximum reflectance in the primary and secondary regions of higher reflectance varied between 28% and 58% and 3% and 11% of the light source, respectively. Primary and secondary regions of higher reflectance were higher in the coverts than in the other body patches. Maximum reflectances in the visible and UV parts of the spectra, as well as reflectances in the short ($R_{340\text{-}min}$) and long ($R_{min\text{-}700}$) wavelengths, covaried positively ($r > 0.53$, $P < 0.05$). The general shape and location of the reflectance minimum, together with the high correlation between primary and secondary reflectance regions, suggests that these spectra are produced by carotenoid pigments, consistent with studies undertaken in other passerine species, for example tanagers (Bleiweiss 2004b, 2005).

Two-way ANCOVAs performed on the individual variables of the spectra and using year of capture as a covariate (test of parallelism, Fs ≤ 3.4, $P \geq 0.04$, nonsignificant after Bonferroni correction), followed by Scheffé contrasts for those variables that differed significatively among species, revealed the following patterns (Table 1, Fig. 2): 1) *S. loyca* and *S. defilippii* had brighter (higher $R_{340–700}$) coverts ($P = 0.001$) and belly ($P \leq 0.01$) than *S. superciliaris*, 2) *S. loyca* had a higher λR_{min} than *S. superciliaris* in the coverts ($P = 0.004$), while differences in the throat were significant only during the nonbreeding season ($P = 0.03$), 3) *S. defilippii* and *S. loyca* typically had higher reflectance in the short wavelength segment of the spectrum. This resulted in a lower $R_{contrast}$ for *S. defilippii* and *S. loyca* than for *S. superciliaris* ($R_{contrast}$ also tended to be lower for *S. defilippii* than for *S. loyca*). In particular, *S. defilippii* had lower $R_{contrast}$ than the other two species in the belly and chest ($P \leq 0.005$ in all cases). *S. defilippii* also showed a lower $R_{contrast}$ in the throat than *S. superciliaris* ($P = 0.006$), but only in the nonbreeding season. Finally, in the coverts *S. loyca* had a lower $R_{contrast}$ than *S. defilippii* ($P < 0.001$), and both species had lower $R_{contrast}$ than *S. superciliaris* ($P < 0.001$), and 4) The secondary segment of reflectance was centered on a lower λR_{UVpeak} in

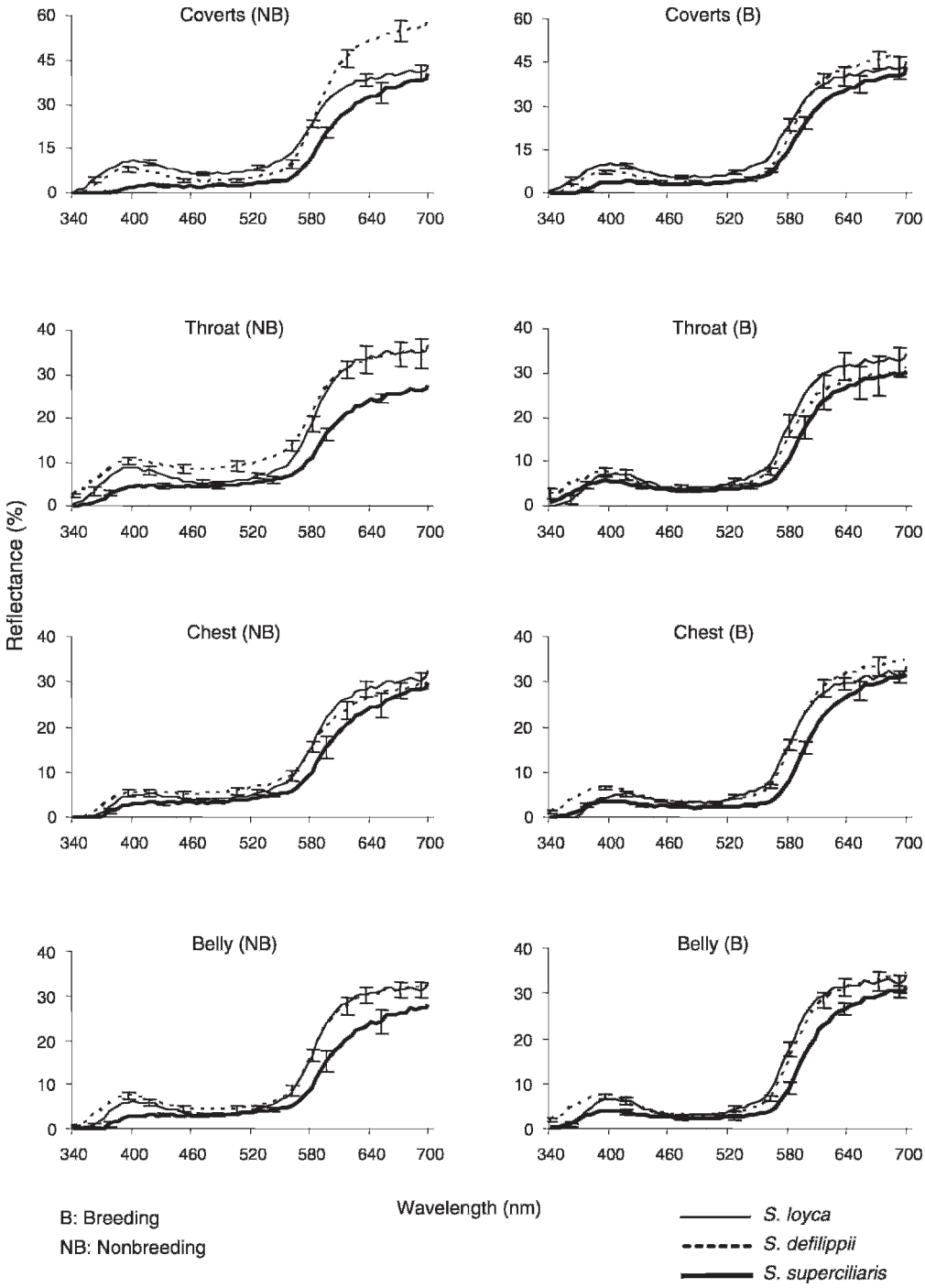

FIGURE 2. Reflectance spectra for the plumage of males of *Sturnella superciliaris* ($n = 12$), *S. defilippii* ($n = 16$, except for throat where $n = 8$), and *S. loyca* ($n = 15$), in the breeding and nonbreeding seasons (B and NB, respectively). Each spectrum represents the mean reflectance ± SE (error bars). Note the different *y*-axis scale for the coverts graphs. These spectra show significant color differences among species and between seasons.

TABLE 1. Species differences in plumage spectral variables for *Sturnella superciliaris, S. defilippii*, and *S. loyca* in each body region (coverts, throat, chest, and belly). See Figure 1 for definitions and illustration of the spectral variables. Values represent means ± SD. Statistics represent the main effect of species in two-way ANCOVAs, with species and season as main factors and year of capture as a covariate. The effect of season is shown in Table 2. Significant *P*-values after Bonferroni correction are denoted with an asterisk.

Variable	Body region	*S. superciliaris*	*S. defilippii*	*S. loyca*	df	*F*	*P*
$R_{340–700}$	Coverts	955 ± 224	1317 ± 243	1325 ± 251	2, 37	11.6	< 0.001*
	Throat	797 ± 217	1120 ± 302	1062 ± 310	2, 29	3.6	0.04
	Chest	730 ± 160	937 ± 183	900 ± 144	2, 37	5.3	0.009
	Belly	724 ± 174	963 ± 209	947 ± 165	2, 37	6.9	0.003*
λR_{50}	Coverts	584.62 ± 4.30	587.11 ± 1.96	588.34 ± 4.97	2, 37	4.8	0.01
	Throat	584.00 ± 10.39	583.11 ± 8.14	587.92 ± 3.80	2, 29	3.7	0.04
	Chest	587.56 ± 5.83	586.98 ± 8.70	586.77 ± 4.36	2, 37	1.3	0.28
	Belly	588.75 ± 7.50	591.76 ± 4.60	587.42 ± 3.21	2, 37	2.3	0.12
λR_{min}	Coverts	468.75 ± 8.59	474.10 ± 3.60	477.57 ± 7.58	2, 37	11.6	0.001*
	Throat	470.01 ± 19.92	469.36 ± 15.77	475.88 ± 7.04	2, 29	2.4	0.11
	Chest	477.04 ± 9.19	474.66 ± 17.56	473.25 ± 8.67	2, 37	2.4	0.10
	Belly	477.50 ± 15.23	484.33 ± 8.94	476.93 ± 1.80	2, 37	2.7	0.08
λR_{UVpeak}	Coverts	422.17 ± 1.45	398.69 ± 7.01	404.83 ± 8.04	2, 37	42.2	< 0.001*
	Throat	407.08 ± 11.85	400.59 ± 6.00	407.51 ± 8.92	2, 29	3.8	0.04
	Chest	418.35 ± 21.43	402.17 ± 12.90	416.57 ± 6.07	2, 37	10.0	< 0.001*
	Belly	411.27 ± 13.40	397.16 ± 6.44	404.49 ± 9.67	2, 37	9.6	< 0.001*
$R_{contrast}$	Coverts	0.90 ± 0.04	0.82 ± 0.05	0.71 ± 0.06	2, 37	43.2	< 0.001*
	Throat	0.77 ± 0.08	0.68 ± 0.06	0.74 ± 0.06	2, 29	5.5	0.01
	Chest	0.83 ± 0.04	0.76 ± 0.05	0.82 ± 0.06	2, 37	5.7	0.007
	Belly	0.83 ± 0.04	0.72 ± 0.05	0.79 ± 0.05	2, 37	15.9	< 0.001*

S. defilippii and *S. loyca* than in *S. superciliaris* (and it tended to be lower in *S. defilippii* than in *S. loyca*). In particular, *S. defilippii* exhibited a lower λR_{UVpeak} than *S. superciliaris* year-round in the coverts ($P < 0.001$) and belly ($P < 0.001$), but only during the nonbreeding season in the throat ($P = 0.04$) and chest ($P < 0.001$). In turn, *S. loyca* showed a lower λR_{UVpeak} than *S. superciliaris* in the coverts ($P < 0.001$) all year long and in the throat during the nonbreeding season ($P = 0.03$), but a higher λR_{UVpeak} than *S. defilippii* in the chest ($P = 0.005$).

Comparisons between seasons (Table 2, Fig. 2) showed that: 1) Total reflectance and $R_{contrast}$ did not change for any of the species, 2) λR_{min} was higher in breeding than in nonbreeding plumage for the belly in all three species, 3) λR_{50} was higher in breeding plumage in the throat for *S. superciliaris* ($P = 0.003$) and in the chest for *S. defilippii* ($P < 0.001$), and 4) the λR_{UVpeak} was lower in the breeding compared to the nonbreeding season in the throat ($P = 0.001$), chest ($P < 0.001$), and belly ($P = 0.004$) for *S. superciliaris* and in the belly for *S. defilippii* ($P = 0.004$).

Quantification of the color differences between *Sturnella* species and between plumages in the breeding and nonbreeding seasons using the perceptual model of Vorobyev and Osorio (1998) corroborated all plumage differences described above, with the exception of color differences between seasons in the coverts of *S. defilippii* (Table 3, 4). Most ΔS values of interspecific color differentiation and intraspecific seasonal color differentiation were considerably higher than the threshold for avian visual discrimination. Notably, coloration of the coverts showed large differences among species (Table 3) and, in general, *S. superciliaris* exhibited more change in coloration from breeding to nonbreeding plumages (Table 4). This resulted in *S. superciliaris* differing more from *S. defilippii* in the nonbreeding than in the breeding season in all plumage areas (repeated measures Student *t*-test = 3.7, $P < 0.05$), and in *S. superciliaris* showing larger differences in the nonbreeding than in the breeding season in relation to *S. loyca* in all plumage areas but the chest.

DISCUSSION

PATTERNS OF COLOR VARIATION AND THEIR POSSIBLE CAUSES

There are striking differences in the plumage of sympatric species of red-breasted meadowlarks

TABLE 2. Differences in plumage spectral variables between the nonbreeding and breeding seasons for *Sturnella superciliaris, S. defilippii*, and *S. loyca* for each body region (coverts, throat, chest, and belly). See Figure 1 for definitions and illustration of the spectral variables. Values represent means ± SD. Statistics represent the main effect of season in two-way ANCOVAs, with species and season as main factors and year of capture as a covariate. The effect of species is shown in Table 1. Significant *P*-values after Bonferroni correction are denoted with an asterisk.

Variable	Body region	Nonbreeding season	Breeding season	df	*F*	*P*
$R_{340–700}$	Coverts	1245 ± 292	1200 ± 289	1, 37	0.0	0.83
	Throat	1057 ± 280	916 ± 319	1, 29	3.6	0.13
	Chest	849 ± 183	879 ± 184	1, 37	0.1	0.79
	Belly	878 ± 203	900 ± 216	1, 37	0.0	0.96
λR_{50}	Coverts	586.44 ± 5.20	587.14 ± 3.13	1, 37	4.8	0.01
	Throat	581.65 ± 7.33	589.09 ± 6.27	1, 29	16.6	< 0.001*
	Chest	582.68 ± 6.23	590.23 ± 4.67	1, 37	27.8	< 0.001*
	Belly	587.13 ± 5.78	591.04 ± 4.59	1, 37	7.8	0.008
λR_{min}	Coverts	472.54 ± 10.19	474.74 ± 10.19	1, 37	8.2	0.001*
	Throat	465.17 ± 14.16	479.19 ± 11.37	1, 29	14.6	0.001*
	Chest	465.03 ± 12.18	481.89 ± 7.10	1, 37	53.4	< 0.001*
	Belly	474.76 ± 11.04	483.51 ± 7.74	1, 37	12.7	0.001*
λR_{UVpeak}	Coverts	407.50 ± 11.31	407.30 ± 11.94	1, 37	0.0	0.86
	Throat	408.69 ± 9.67	403.03 ± 9.12	1, 29	11.7	0.002*
	Chest	421.16 ± 16.72	404.90 ± 10.97	1, 37	28.9	< 0.001*
	Belly	408.06 ± 11.93	400.48 ± 9.72	1, 37	9.3	< 0.004*
$R_{contrast}$	Coverts	0.80 ± 0.11	0.81 ± 0.07	1, 37	0.1	0.74
	Throat	0.74 ± 0.09	0.74 ± 0.06	1, 29	0.3	0.58
	Chest	0.82 ± 0.05	0.79 ± 0.06	1, 37	4.3	0.05
	Belly	0.79 ± 0.07	0.77 ± 0.06	1, 37	2.4	0.13

in both the UV and human-visible parts of the spectrum. These differences persist year-round and include not only brightness but also spectral shape. In particular, *S. loyca* and *S. defilippii* show brighter red patches and a higher reflectance in the UV region of the spectrum than *S. superciliaris*. Short (1968), based on human visual perception, also reported differences in the red ventral coloration of these species, noting that *S. defilippii* is redder than the pinker *S. loyca*. Our finding of higher values for hue in the former species supports his conclusion, although the differences were nonsignificant.

In addition to interspecific differences, we found significant differences between seasonal plumages within species, especially in *S. superciliaris* and *S. defilippii*. Short (1968) reasoned that the seasonal change in *S. loyca* and *S. defilippii* plumage color was due to fading of the brown tips of their red feathers. The same process may also account for the change in hue in the red plumage patches that we measured (as estimated by longer λR_{50} and λR_{min}). Abrasion of the brown tips would be predicted to expose the red-pigmented bases of the feathers, shifting the spectrum toward longer visible and shorter, near-UV, wavelengths. A test of abrasion as a cause of seasonal change in color in *Sturnella* plumages will require a microanatomical study of their feathers (Willoughby et al. 2002).

The plumage color differences among *Sturnella* species that we have documented here should be visually detectable by these birds, because the color distances in avian perceptual space largely exceeded the threshold for color discrimination, especially in the coverts, suggesting that this feather patch, which is conspicuously exhibited during flight displays by *S. superciliaris* and *S. defilippii*, may be of great importance as a species-specific marker. We also found that changes in color between breeding and nonbreeding plumages were above the discrimination threshold (Vorobyev et al. 1998, Vorobyev 2003, Siddiqi et al. 2004, Eaton 2005). These patterns of seasonal change in color are a consequence of the pattern of molt and wear of the feathers. *Sturnella* species only have one complete postreproductive molt (Jaramillo and Burke 1999). This molt results in larger differences in avian visual color space between *S. superciliaris* and the other two species than between *S. defilippii* and *S. loyca*.

TABLE 3. Color differentiation (ΔS) between *Sturnella superciliaris, S. defilippii*, and *S. loyca* during the breeding and nonbreeding seasons. ΔS represents the relative distance between species for each body region in avian perceptual color space. The units of ΔS are jnd (just noticeable differences), with 1.0 jnd being the threshold value for discrimination of colors.

Body region Species	Breeding season ΔS	Nonbreeding season ΔS
Coverts		
S. defilippii vs. *S. loyca*	7.1	9.4
S. defilippii vs. *S. superciliaris*	9.7	17.6
S. loyca vs. *S. superciliaris*	13.4	23.6
Throat		
S. defilippii vs. *S. loyca*	6.7	4.9
S. defilippii vs. *S. superciliaris*	3.2	8.3
S. loyca vs. *S. superciliaris*	5.0	6.0
Chest		
S. defilippii vs. *S. loyca*	11.5	4.7
S. defilippii vs. *S. superciliaris*	6.4	7.8
S. loyca vs. *S. superciliaris*	8.2	3.7
Belly		
S. defilippii vs. *S. loyca*	7.3	5.0
S. defilippii vs. *S. superciliaris*	6.4	13.2
S. loyca vs. *S. superciliaris*	4.1	8.7

TABLE 4. Plumage color differentiation (ΔS) between the breeding and nonbreeding seasons, within *Sturnella superciliaris, S. defilippii*, and *S. loyca*. ΔS represents the relative distance between the breeding and nonbreeding season for each body region in avian perceptual color space. The units of ΔS are jnd (just noticeable differences), with 1.0 jnd being the threshold value for discrimination of colors.

Species	Body region	Breeding vs. nonbreeding season, ΔS
S. superciliaris	Coverts	8.9
	Throat	8.4
	Chest	10.7
	Belly	11.1
S. defilippii	Coverts	1.0
	Throat	6.9
	Chest	10.0
	Belly	5.1
S. loyca	Coverts	2.4
	Throat	3.5
	Chest	2.9
	Belly	1.1

Subsequently, feather wear of *S. superciliaris* produces a greater similarity to the plumages of the other two species (in particular to *S. defilippii*), a phenomenon clearly noticeable in the breeding season. This result is interesting, because, if color signals are important for conspecific mate choice, plumage differences should be larger in the breeding season. However we cannot exclude other possible explanations, such as color convergence facilitating interspecific aggression and promoting interspecific territoriality, which has been documented between *S. superciliaris* and *S. defilippii* (Gochfeld 1979).

Several studies have shown that the spectral properties of plumage in museum specimens may change as a consequence of plumage fading with time (Endler and Théry 1996, Hausmann et al. 2003). However, the results presented here regarding interspecific and seasonal variation in red plumage patches should not be biased by this phenomenon, as year of capture was included as a covariate in the statistical analyses. A recent study by McNett and Marchetti (2005) showed differences in carotenoid patches between living and museum specimens. They found that both hue and chroma decreased in the spectra of museum specimens as a consequence of changes in color at shorter wavelengths. This result suggests that the plumage of museum specimens may be unrepresentative of natural avian colors, especially for variables in the UV region. Obviously, we must be aware of this possibility; however, it is possible that differences between live and museum specimens might also be explained by differences in measurement error between live birds and study skins (e.g., because of subtle movements of live birds while taking spectral readings, or the arrangement of the feathers of live birds in the hand). These possibilities require further investigation across all studies of avian coloration.

In general, noniridescent red feathers result from carotenoid pigmentation (Gray 1996, Mahler et al. 2003, Bleiweiss 2005). Carotenoid pigments have a distinctive absorption band between 400 and 500 nm (Hudon and Brush 1992, Ryan et al. 1994, Shawkey and Hill 2005). This results in a bimodal reflectance spectrum with a primary band of reflectance in the long wavelength region and a secondary one in the near-UV portion of the spectrum, strongly reminiscent of the patterns we obtained for some red-breasted meadowlarks. Thus, pending detailed biochemical study, we tentatively assume that the red plumage patches in

meadowlarks are indeed carotenoid based. The presence and magnitude of the secondary region of reflectance varied between species; it was evident in *S. loyca* and *S. defilippii*, but almost absent in *S. superciliaris*. Such interspecific variation has also been detected in tanagers (Bleiweiss 2004b, 2005). Variation in the presence of the secondary segment of reflectance may be caused by several nonexclusive factors, including: 1) the type of carotenoid pigment present and its concentration, with higher concentration reducing the secondary UV peak; 2) the presence of small quantities of melanins, pigments that absorb light differentially in the near-UV part of the spectrum; and 3) subtle modifications in the microstructure of feathers that change the amount of short wavelengths reflected (see Bleiweiss [2004b] and Andersson and Prager [2006] for a detailed discussion of these possibilities). We do not yet know which of these explanations accounts for variation in near-UV reflectance among the species we examined, but we expect a higher concentration of carotenoids in *S. superciliaris* compared to *S. defilippii* and *S. loyca*, and the presence of more melanin in the former compared to the latter two species. This second prediction is supported by the observation that colors other than red in the plumage of *S. superciliaris* are notably darker than in *S. defilippii* and *S. loyca* (with the exception of the white brow).

DO COLOR DIFFERENCES IN RED-BREASTED MEADOWLARKS PLAY A ROLE IN SPECIES RECOGNITION?

Obviously, the question of whether interspecific variation in color among closely related sympatric species of meadowlarks plays a role in reproductive isolation cannot be answered just by quantifying differences in reflectance spectra. Such a conclusion will require discrimination and preference tests. However, the fact that almost no interspecific hybrids or hybrid pairings have been detected to date (Gochfeld 1975) is indirect evidence suggesting that red-breasted meadowlarks can efficiently discriminate between species. The absence of interspecific territoriality and aggression between *S. loyca* and the other two species, despite territory overlap, lends further support to this hypothesis (Gochfeld 1975, 1979). However, *S. superciliaris* and *S. defilippii* maintain nonoverlapping territories by means of interspecific aggression. This aggression has been attributed to their similarities in both plumage and behavior, which are not a consequence of character convergence but rather of a close phylogenetic relationship (Gochfeld 1979).

Color differences between closely related species of meadowlarks might have evolved as a consequence of several nonexclusive processes, such as habitat differences in light environment, sexual selection, or simply random drift along independent evolutionary histories. Given the degree of similarity in the use of open habitats by these three species, together with their degree of sympatry and syntopy, the former explanation is likely not tenable. In contrast, sexual selection is considered a potent cause of species divergence (Andersson 1994, Panhuis et al. 2001) and is likely to have operated in *Sturnella* given their high degree of sexual dimorphism in color, size, and behavior (Short 1968, Gochfeld 1975, 1979, Jaramillo and Burke 1999). Because of this, we tentatively interpret interspecific differences in red-colored patches among *Sturnella* species as the result of sexual selection. However, we cannot exclude the possibility that selection for species recognition has played a role in shaping interspecific differences in color. The presence of reproductive character displacement, a pattern consisting of an increase in differences between sympatric compared to allopatric populations of different species, would support this hypothesis (Panhuis et al. 2001). Such a pattern has been shown in the plumage of a few species of birds (Andersson 1994). We did not plan to test for reproductive character displacement, because most specimens we measured came from areas of sympatry, and small sample sizes precluded comparisons between sympatric and allopatric populations. In addition, detecting such a pattern would be challenging, because: 1) the distribution of *S. defilippii* is completely within that of *S. superciliaris*, and 2) many sites are currently occupied only by *S. superciliaris*, either due to range expansion or to the very recent reduction in the distribution of *S. defilippii* (Tubaro and Gabelli 1999). Moreover, *S. loyca* is a recent invader of the southern pampas (Gochfeld 1975), thus its sympatry with the other species of red-breasted

meadowlarks may be too recent to have detectable evolutionary consequences.

Of course, red-breasted meadowlarks not only differ in their red-colored patches, as shown in our study, but also in song and call repertoires and associated behaviors (e.g., flight displays; Gochfeld 1975). Future work should assess the relative importance of interspecific variation in acoustic and visual signals for species recognition and male–male or male–female interactions with the simultaneous use of playbacks and decoys with known reflectances.

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