



Age-dependent relationships between coloration and reproduction in a species exhibiting delayed plumage maturation in females

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Males of many bird species exhibit delayed plumage maturation (DPM), a condition in which young individuals display an immature plumage. Several adaptive hypotheses have been suggested for the signaling utility of DPM in males. Tree swallows *Tachycineta bicolor*, however, are one of the few bird species to exhibit DPM in females, but not in males. Few studies have focused on the age-dependent signaling function of female plumage traits due to the uncommon nature of DPM in females. Therefore, we used reflectance spectrometry and scanning electron microscopy of sub-adult (melanin-based brown) and adult (iridescent-blue structural) female tree swallows to characterize plumage coloration. Next, we asked whether variation in plumage coloration in females reflects condition and reproductive performance between and within age classes. We found that older females were in better body condition and laid eggs earlier in the season compared to young females; however, average egg mass and reproductive success (number of offspring fledged and offspring condition) did not differ between age classes. There were significant relationships indicating that young females with more-ornamented (darker brown melanin) plumage laid smaller eggs, but hatched eggs earlier in the season leading to nestlings in better condition compared to less-ornamented young females. Older females that were more ornamented (brighter, greater blue chroma, and lower hues in iridescent plumage) laid heavier eggs, but ornamentation was negatively associated with immune function, health, and reproductive success. Together, these data suggest that female ornamentation reflects reproductive performance and that there are complicated relationships between plumage coloration, condition, and reproductive performance that ultimately influence reproductive success.

Variation in sex-specific ornamental traits is often explained by sexual selection; males that display more-exaggerated traits compete better for mates and consequently experience higher reproductive success (Andersson 1994). Although the significance of male plumage coloration has been well studied, little research has addressed the importance of female plumage coloration in signaling quality and reproductive investment (reviewed by Amundsen and Parn 2006). The most widely held explanation is that exaggerated and variable expression of ornamentation within females is a byproduct of sexual selection acting on male traits (Amundsen 2000a, b, Amundsen and Parn 2006). However, several recent studies challenged this idea with evidence of sexual selection acting directly on ornamental female traits (*Luscinia svecica*, Amundsen et al. 1997; *Cyanoliseus patagonus*, Masello and Quillfeldt 2003; *Sialia sialis*, Siefferman and Hill 2005; *Petronia petronia*, Griggio et al. 2010a; *Tyto alba*, Roulin et al. 2010; *Cyanistes caeruleus*, Doutrelant et al. 2011). Female Eurasian dotterels *Charadrius morinellus* with brighter plumage, for example, compete better in same-sex interactions and experience greater reproductive success (Owens et al. 1994).

When ornamental traits reliably signal dominance, aggressive interactions can be avoided, thus, maintaining social stability during periods of intense conspecific

competition (West-Eberhard 1979, 1983). Delayed plumage maturation (DPM) is a life-history strategy in which reproductively viable young individuals display 'sub-adult' plumage. While there is no consensus for the function of DPM, it is most commonly thought to benefit first time breeders by reducing the likelihood that older individuals will initiate agonistic interactions (Lyon and Montgomerie 1986, Hawkins et al. 2012). DPM is frequently seen among males, but is a rare phenomenon in females. To the best of our knowledge, the only North American bird species in which female DPM has been described are tree swallows *Tachycineta bicolor* (Lozano and Handford 1995) and hooded warblers *Wilsonia citrina* (Morton 1989). In tree swallows, after-second year (ASY) females exhibit iridescent blue plumage; whereas, second year (SY) females, or first-time breeders, have dull non-iridescent brown plumage (Hussell 1983).

Female tree swallows compete aggressively for limited nesting cavities and only the most aggressive females attain a cavity, suggesting that sexual selection acts on female aggressiveness (Rosvall 2008). Older females tend to be in better body condition (Ardia and Clotfelter 2007) and out-compete younger females for breeding sites (Lozano and Handford 1995). Interestingly, higher body condition in female tree swallows is positively correlated

with aggressiveness (Rosvall 2011). Therefore, it is likely that DPM in female tree swallows functions as a signal of subordination. For DPM to signal subordination, SY females should not only receive less aggression, but also be less aggressive (Rosvall 2008). Stutchbury and Robertson (1987) provided evidence that SY females receive less aggression from male, but not from adult female tree swallows in naturally occurring intrusions. However, when adult pairs were simultaneously presented with SY and ASY female models and the male was unresponsive, adult females attacked the ASY model more often than the SY model (Stutchbury and Robertson 1987). This suggests that ASY females will respond aggressively to any nest intruder, but if given a choice, they perceive other ASY females to be a greater threat than SY females. Concerning female aggressiveness, Rosvall (2008) showed that SY and ASY females respond equally aggressively when individually presented with a SY model; however, future studies should determine if SY and ASY females also respond equally aggressively to an ASY model. If DPM is a signal of subordination, it is likely that SY females would respond with less aggression toward ASY females.

Although female tree swallows are known to display DPM and female age has been shown to predict reproductive performance (Hussell 1983, Wheelwright and Schultz 1994, Robertson and Rendell 2001), little research has focused on how within-age-class variation in plumage coloration signals condition and predicts reproductive performance. A study of SY female tree swallows showed that brighter females with a greater percentage of iridescent feathers laid heavier eggs earlier in the season in a PCB-contaminated environment (McCarty and Secord 2000). A study focusing on plumage variation within ASY female tree swallows found that brighter females laid heavier eggs and females with bluer hues fledged more offspring (Bitton et al. 2008).

Here we test relationships between plumage coloration, condition, and reproductive performance within SY and ASY female tree swallows. We also test the relationship between plumage coloration and attributes of health (cell-mediated immunity and hematocrit) in ASY females. In tree swallows, few studies have focused on the age-dependent signaling function of plumage coloration within age classes (McCarty and Secord 2000, Bitton et al. 2008); therefore, little is known about why variation in female plumage coloration within age classes exists and what it may indicate. Our objectives were to assess differences between SY and ASY female condition, plumage, and reproductive performance; however, more importantly, we sought to determine if plumage ornamentation reflects reproductive performance within each age class. Moreover, we characterize the basis of plumage reflectance in SY and ASY females using reflectance spectrometry and microscopy.

Methods

Study site and general procedure

Tree swallows are socially monogamous passerines that readily breed in nest boxes in open fields and marshes where

they forage aerially for insects (Robertson et al. 1992). Both males and females defend their nest cavity from conspecifics, other species of cavity nesting birds, and potential nest predators (Robertson et al. 1992). We monitored 230 nest boxes daily to determine clutch-initiation dates from April to July in 2009 and 2010 in Watauga County, North Carolina (36°12'41"N, 81°40'7"W). Upon clutch completion, we measured clutch mass (± 0.1 g) and divided by clutch size to determine average egg mass. We captured females during early incubation and measured wing length (± 0.1 mm) and mass (± 0.1 g). We created an index of female body condition using residuals of a regression of mass on wing length ($R^2 = 0.10$, $F_{(1,143)} = 15.42$, $p < 0.001$; Brown 1996). We used age classifications proposed by Hussell (1983), in which female tree swallows can be aged with a 95% accuracy using the percent iridescence of feathers on the rump, back, and head. Females were designated as SY if up to 50% of their body plumage on the head, back or rump was brown or ASY if more than 90% was iridescent (Hussell 1983). An intermediate age classification was not used because they can not be accurately placed in an age class (Hussell 1983); we excluded five females that displayed body plumage with 50–90% iridescence.

In a subset of ASY females ($n = 13$), we used the antigen phytohaemagglutinin (PHA; Sigma-Aldrich, St Louis, MO, USA) test to measure cell-mediated immune response. As a reference, we measured thickness of the right wing web using a micrometer (± 0.05 mm; Smits et al. 1999). We then injected 20 mg of PHA in a 50 μ L phosphate buffered saline (PBS) into the right wing web. We re-measured wing web thickness upon re-capture 24 h after PHA injection. Each measurement was performed consecutively three times and the average was used. We calculated repeatability of pre- and post-measurements using the intraclass correlation coefficient (Lessells and Boag 1987). The PHA index was established as the percent change of wing web thickness from pre- to post-injection. Also, in a subset of ASY females ($n = 20$), we collected ~ 150 μ L of blood by veinpuncture of the cutaneous ulnar vein. We spun the blood to separate plasma and red blood cells (RBC) and measured hematocrit as the ratio of packed RBC to total blood volume (Brown 1996).

Throughout the breeding season we monitored clutches and recorded hatch date. At age 8 d post-hatch (hatch day = 1) we banded nestlings and at age 14 d post-hatch (prior to fledging; Robertson et al. 1992) we measured mass (± 0.1 g) and wing length (± 0.1 mm). We then calculated an index of nestling body condition using the residuals from a linear regression of mass on wing length at day 14 post-hatch ($R^2 = 0.10$, $F_{(1,554)} = 62.80$, $p < 0.001$; Brown 1996).

Plumage analysis

Upon capture, we plucked 10 rump feathers from females for spectrometric analyses. The feathers were placed on black paper so they mimicked how they would naturally lay on the bird. We measured spectral reflectance with an Ocean Optics S2000 spectrometer (range 250–880 nm: Dunedin, FL, USA), illuminated with both UV (deuterium bulb) and visible (tungsten-halogen bulb) light sources,

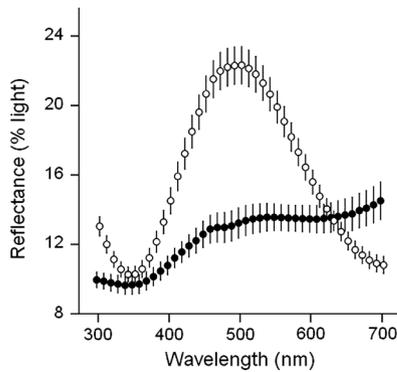


Figure 1. Reflectance spectra (mean \pm 95% CI) of rump feathers of second year (closed circles, $n = 44$) and after-second year (open circles, $n = 62$) female tree swallows.

and a WS-2 white standard (Lapsphere, North Sutton, NH, USA). We used a bi-furcated micron fiber optic probe at a 90° angle 1 mm from the feather surface. We measured plumage reflectance from five randomly chosen points on each sample using OOIbase software; each reading represented an average of 20 scans. For blue iridescent plumage (Fig. 1), we summarized reflectance data using three common methods: brightness, hue, and blue chroma. We calculated brightness (the total light reflected by feathers) as the average of the summed reflectance from 300 to 700 nm and hue as the wavelength at peak reflectance (Siefferman and Hill 2005). We calculated blue chroma as the proportion of the total reflectance in the blue range to the total reflectance of the entire spectrum ($R_{400-512 \text{ nm}}/R_{300-700 \text{ nm}}$; Bitton et al. 2008). For blue iridescent plumage, we used principal components analysis to reduce the three color measures to principal component 1 (PC 1) that explained 68.13% of the variation (brightness loading, 0.534; hue loading, -0.925 ; blue chroma loading, 0.950). ASY females with high positive PC 1 exhibited brighter, greater blue chroma, and shorter-wavelength hues. The brown plumage of SY females is achromatic, so we used only brightness to characterize it (Fig. 1).

We sampled feathers from three brown SY and three iridescent ASY females to characterize morphology of feather barbules. We mounted feathers on aluminum pin stubs (Electron Microscopy Sciences, Hatfield, PA, USA) with carbon tape and sputter-coated them with gold using a Denton Vacuum Desk III Sputter Coater (Moorestown, NJ, USA). We then photographed the barbules nearest the distal fork of the rachis of each feather using a FEI Quanta 200 Environmental Scanning Electron Microscope (Hillsboro, OR, USA).

Statistical analyses

Data were normally distributed (Shapiro–Wilk test, $p > 0.2$) and SPSS (ver. 17.0; SPSS, Chicago, IL) was used to analyze data; all statistical tests were two-tailed. All means are followed by the standard error unless otherwise stated. Three-dimensional figures were designed in SigmaPlot (ver. 11.0; Systat Software, San Jose, CA) and a neighborhood average function was used to smooth data. We tested

for an effect of year (2009 vs 2010) on all female and nestling parameters, because we failed to detect significant differences (all $t < 1.5$, all $p > 0.08$), we combined data from both years. Of the females that bred both years ($n = 22$), only their first breeding attempt was used to avoid pseudo-replication.

Because past research indicates that sub-adult and adult female tree swallows differ in morphology, condition, and reproductive investment (De Steven 1978, Hussell 1983, Lozano and Handford 1995, Robertson and Rendell 2001, Ardia and Clotfelter 2007), we first tested for effects of age on morphological and reproductive characteristics using independent t -tests. For all comparisons, we tested for equality of variance using Levene's test; when variance differed significantly we used the Welch–Satterthwaite t -test. Age significantly influenced many female characteristics, so we separated SY and ASY females in further analyses.

Reproductive parameters, such as timing of breeding (De Steven 1978, Wheelwright and Schultz 1994, Lozano and Handford 1995, Winkler and Allen 1996, Svensson 1997, Robertson and Rendell 2001) and egg mass (Wiggins 1990, Whittingham et al. 2007) are known to be important predictors of reproductive success. Therefore, we used a linear regression model to investigate associations between hatch date and plumage coloration, and a backwards multiple regression model to investigate associations between average egg mass and the variables hatch date and plumage coloration within each age class.

Next, also using backwards multiple regression models, we tested whether reproductive success was influenced by plumage coloration, body condition, hatch date, and average egg mass. Our measures of reproductive success included a) brood average nestling body condition and b) number of offspring fledged. We included all first order interactions as independent variables and centered all variables to avoid multicollinearity (Aiken and West 1991). We re-tested our models using forward elimination procedures to assure the accuracy of our conclusions and found that they were unchanged. The female body condition index was not significant in any of the models tested and was removed from all analyses and discussion of within age class variation.

Results

SY and ASY females significantly differed in body condition, color parameters, and hatch date. ASY females had a significantly higher body condition index (0.15 ± 0.11) than SY females (-0.30 ± 0.12 ; $t_{2,137} = -2.57$, $p = 0.01$). ASY females also had significantly earlier hatch dates (145.65 ± 0.37) than SY females (152.54 ± 1.21 ; $t_{2,45,35} = 5.46$, $p < 0.001$). Based on our age criteria, ASY females were brighter ($0.15\% \pm 0.003$) with lower hues ($499.57 \text{ nm} \pm 3.23$) and greater blue chroma (0.34 ± 0.003) than SY females (brightness: $0.13\% \pm 0.004$, $t_{2,104} = -4.04$, $p < 0.001$; hue: $653.50 \text{ nm} \pm 10.59$, $t_{2,51,04} = 13.90$, $p < 0.001$; blue chroma: 0.27 ± 0.004 , $t_{2,104} = -14.10$, $p < 0.001$). However, we found no significant relationship between female age and egg mass, nestling body condition index, or number of offspring fledged (all $t < 1.55$, all $p > 0.12$).

The reflectance spectra of SY female tree swallows (Fig. 1) are typical of brown melanin plumage (McGraw 2006) and the reflectance spectra of ASY females (Fig. 1) are indicative of structural coloration (Prum 2006). Microscopy revealed that feather barbules of SY females were sparsely distributed, while ASY feather barbules were flattened and parallel to the feather surface, appeared more densely packed, and showed clear delineation between cells, suggesting they are more conducive to light reflectance and, thus, iridescence (Doucet et al. 2006; Fig. 2a–d). Brightness of SY feather coloration was not significantly associated with percent iridescence ($R^2 = 0.08$, $F_{(1,29)} = 2.32$, $p = 0.14$). However, ASY females with higher percent iridescence had brighter, greater blue chroma, and shorter wavelength hues (greater PC 1; $b = 0.46$) and percent iridescence explained a significant proportion of variance in PC 1 ($R^2 = 0.21$, $F_{(1,47)} = 12.35$, $p = 0.001$).

Reproductive investment was significantly correlated with color parameters within the two age classes. Among SY females, brightness explained a significant proportion of variance in hatch date ($R^2 = 0.27$, $F_{(1,29)} = 10.17$, $p = 0.003$); darker females laid eggs significantly earlier in the breeding season than did brighter females ($b = 0.52$). Brightness was also the only significant predictor of egg mass ($R^2 = 0.26$, $F_{(1,17)} = 5.66$, $p = 0.03$); brighter females laid heavier eggs (Table 1). Among ASY females, those with higher PC 1 scores laid heavier eggs ($R^2 = 0.22$, $F_{(1,34)} = 9.29$, $p = 0.005$; Table 1); however, plumage coloration did not significantly predict hatch date ($R^2 = 0.001$, $F_{(1,43)} = 0.02$, $p = 0.88$).

Reproductive success was also correlated with color parameters within each age class. For SY females, all the predictor variables (hatch date, brightness, and average egg mass) were significant in the final model predicting nestling

body condition ($R^2 = 0.69$, $F_{(3,13)} = 7.28$, $p = 0.01$; Table 1); darker melanic females that laid eggs earlier in the season (Fig. 3a) and laid heavier eggs (Fig. 3b) tended to produce offspring in higher body condition. Within ASY females, of all the predictor variables (hatch date, PC 1, and average egg mass) only PC 1 significantly predicted offspring body condition ($R^2 = 0.16$, $F_{(1,31)} = 5.80$, $p = 0.02$; Table 1); ASY females with high PC 1 scores (brighter, greater blue chroma, and shorter-wavelength hues) produced offspring in lower body condition. Furthermore, brightness, average egg mass, and their interactions with hatch date significantly predicted the number of offspring fledged by SY females ($R^2 = 0.88$, $F_{(4,13)} = 15.77$, $p < 0.001$; Table 1); in general, females that were darker and laid smaller eggs fledged more offspring. However, there was a significant interaction between hatch date and brightness, and hatch date and average egg mass indicating that the strength of the negative relationships varied; the relationship was stronger for females that hatched eggs later ($b = -0.48$, brightness; $b = -0.68$, average egg mass) than those that hatched eggs earlier in the season ($b = -0.44$, brightness; $b = -0.23$, average egg mass). No predictor variables significantly predicted number of offspring fledged by ASY females ($R^2 = 0.04$, $F_{(1,31)} = 1.28$, $p = 0.27$).

Additionally, we found evidence that plumage coloration is negatively correlated with measures of immune and health function. ASY females with higher PC 1 scores (brighter, greater blue chroma, and shorter-wavelength hues) exhibited a poorer cell-mediated immune response ($R^2 = 0.36$, $F_{(1,12)} = 6.15$, $p = 0.03$; Fig. 4a) and lower hematocrit scores ($R^2 = 0.30$, $F_{(1,19)} = 7.73$, $p = 0.01$; Fig. 4b). The pre- and post-PHA measurement were highly repeatable with intraclass correlation coefficients of 0.97 and 0.96, respectively.

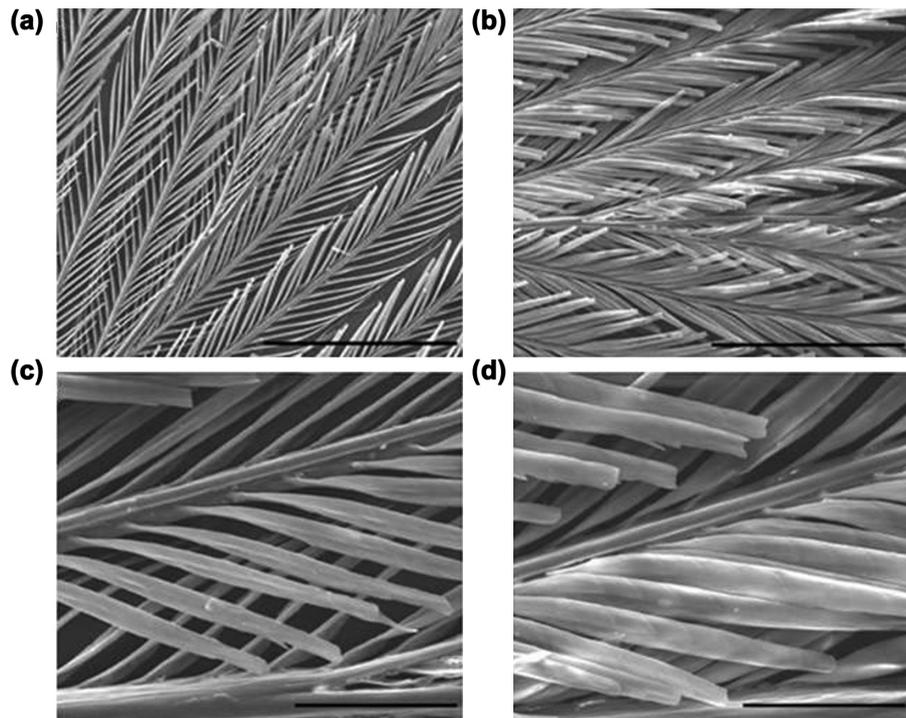


Figure 2. Comparison of rump feathers from second year (a and c) and after-second year (b and d) female tree swallows. Scanning electron microscope images were taken at the distal end of the rachis. Scale bars, 500 μm (a and b, 257 \times) and 100 μm (c and d, 1077 \times).

Table 1. Backward multiple regression models of sources of variation in egg mass (second-year, SY, final model: $R^2 = 0.26$, $F_{(1,17)} = 5.66$, $p = 0.03$; after-second year, ASY, final model: $R^2 = 0.22$, $F_{(1,34)} = 9.29$, $p = 0.005$), brood average nestling body condition at fledging (SY final model: $R^2 = 0.69$, $F_{(3,13)} = 7.28$, $p = 0.01$; ASY final model: $R^2 = 0.16$, $F_{(1,31)} = 5.80$, $p = 0.02$), and number of offspring fledged (SY final model: $R^2 = 0.88$, $F_{(4,13)} = 15.77$, $p < 0.001$) in female tree swallows; separate analyses were performed for each age class. No predictor variables significantly predicted number of offspring fledged for ASY female tree swallows ($R^2 = 0.04$, $F_{(1,31)} = 1.28$, $p = 0.27$). The variables tested in each model were hatch date, egg mass, and color (brightness for SY and PC 1 for ASY females; greater PC 1 represents brighter, greater blue chroma, and shorter wavelength hues) and the interactions between the variables; only hatch date and color were tested in the model explaining variation in egg mass. Removal criteria for all variables and interactions was $p > 0.10$.

Dependent	Age	Model	β	Partial r	t	p
Egg mass (g)	SY	Variables included				
		Brightness (%)	0.51	0.51	2.38	0.03
		Variables excluded				
		Hatch date	-0.10	-0.11	-0.43	0.68
	ASY	Brightness (%) \times hatch date	0.11	0.13	0.52	0.61
		Variables included				
		PC 1 of color	0.47	0.47	3.05	0.005
		Variables excluded				
		Hatch date	0.02	0.02	0.12	0.91
		Hatch date \times PC 1 of color	0.02	0.02	0.12	0.90
Brood body condition	SY	Variables included				
		Hatch date	-0.50	-0.62	-2.47	0.033
		Brightness (%)	-0.58	-0.62	-2.49	0.032
		Average egg mass (g)	0.70	0.72	3.30	0.008
		Variables excluded				
		Brightness (%) \times hatch date	-0.01	-0.02	-0.05	0.96
		Brightness (%) \times egg mass (g)	0.01	0.01	0.04	0.97
	ASY	Egg mass (g) \times hatch date	-0.12	-0.17	-0.51	0.62
		Variables included				
		PC 1 of color	-0.40	-0.40	-2.41	0.02
		Variables excluded				
		Egg mass (g)	0.25	0.24	1.35	0.19
		Hatch date	-0.23	-0.26	-1.42	0.17
		Egg mass (g) \times hatch date	-0.13	-0.24	-0.74	0.46
No. offspring fledged	SY	PC 1 \times hatch date	0.09	0.09	0.59	0.62
		Egg mass (g) \times PC 1 of color	0.05	0.06	0.30	0.77
		Variables included				
		Brightness (%)	0.36	0.60	2.25	0.051
		Egg mass (g)	-0.83	-0.84	-4.68	0.001
		Brightness (%) \times hatch date	1.24	0.93	7.35	<0.001
		Egg mass (g) \times hatch date	-1.16	-0.90	-6.07	<0.001
Variables excluded						
Hatch date	-0.07	-0.15	-0.42	0.69		
Brightness (%) \times egg mass (g)	0.26	0.44	1.37	0.21		

Discussion

Our data showed clear distinctions in coloration between SY and ASY female tree swallows. The reflectance spectra indicate that the brown plumage of SY females is indeed melanin-based, though it is still unclear which melanin pigments (eumelanin or pheomelanin) cause the coloration, and the iridescent blue plumage of ASY females is structurally based. Due to these inherent differences, feathers of SY and ASY female tree swallows must be analyzed separately. For example, measures such as brightness fundamentally differ for the two age classes. Darker (less bright) SY females should be considered more ornamented, because swallows with darker melanin-based plumage coloration generally have greater melanin concentrations (McGraw et al. 2004, 2005; but see Crowhurst et al. 2012 for diamond firetails *Stagonopleura guttata*, and Hanssen et al. 2006 for eiders *Somateria mollissima*, in which the lack of melanin is considered more ornamented in females). Whereas, ASY females with a higher

PC 1 (higher brightness, lower hue, and greater blue chroma) should be considered more ornamented, because an increased organization of feather nanostructure should cause greater light reflectance (Dyck 1976, Prum 2006).

Between age class comparisons

We found that although ASY females hatched eggs earlier in the breeding season and exhibited better body condition than SY females, there was no evidence that age influenced egg investment or reproductive success. Previous studies of tree swallows have also failed to find an effect of maternal age on nestling body condition (Wheelwright and Schultz 1994, Ardia and Clotfelter 2007) and egg mass (Wiggins 1990, Wheelwright and Schultz 1994). However, other species have shown an increase in egg size with maternal age and ornamentation (Dreiss and Roulin 2010). Robertson and Rendell (2001) found in a 25 yr study of tree swallows that the number of offspring fledged increases from first-time breeders to middle-aged birds and decreases in older

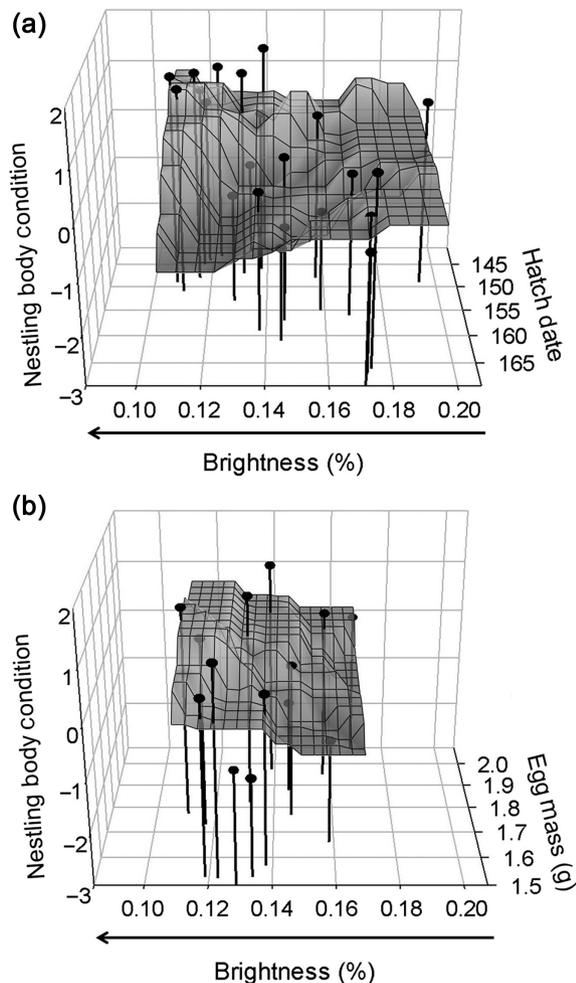


Figure 3. In second year female tree swallows, relationship (a) between brood average nestling body condition at fledging, hatch date (Julian day), and brightness and (b) between brood average nestling body condition at fledging, brightness, and average egg mass. The arrow indicates the direction of increased ornamentation.

birds. Our inability to age birds past ASY could explain why we found no relationship between age and number of offspring fledged.

Like other studies of tree swallows, we found that female age influenced hatch date (De Steven 1978, Wheelwright and Schultz 1994, Lozano and Handford 1995, Winkler and Allen 1996, Svensson 1997, Robertson and Rendell 2001). ASY females laid eggs earlier in the season and bred more synchronously than did SY females. ASY females may breed earlier and more synchronously because they arrive at the breeding site earlier than SY females and, therefore, are less restricted by number of available nesting sites (Lozano and Handford 1995). Alternatively, ASY females may be competitively dominant to SY females and exclude them from early nest selection; our data show that ASY females were in better body condition, and body condition is positively correlated with aggressiveness in female tree swallows (Rosvall 2011). However, if DPM functions as a signal of subordination, SY females should avoid competitions with ASY females. Regardless, SY females likely compete with one another for remaining nest sites.

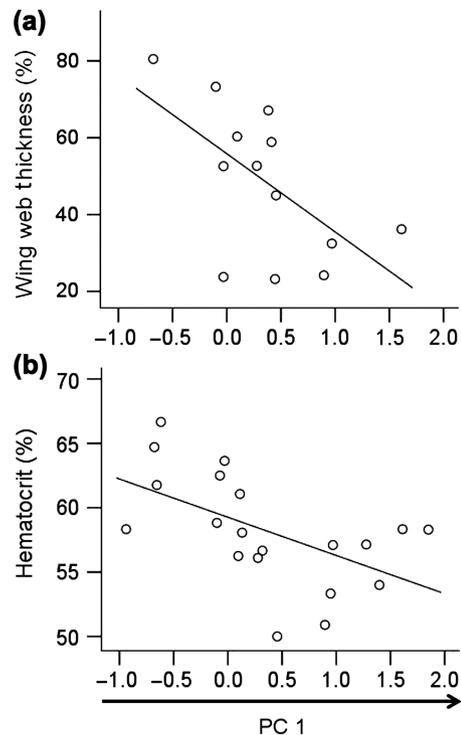


Figure 4. Relationship between after-second year female tree swallow plumage coloration (PC 1) and (a) PHA response as measured in percent change of wing web thickness ($R^2 = 0.36$, $F_{(1,12)} = 6.15$, $p = 0.03$) and (b) hematocrit as measured in percent of red blood cells in total blood volume ($R^2 = 0.30$, $F_{(1,19)} = 7.73$, $p = 0.01$). The arrow indicates the direction of increased ornamentation.

Within age class comparisons

Our data indicate that complex relationships exist between plumage ornamentation and reproductive performance within age classes. Of particular significance were associations found between SY female coloration and reproductive investment, and ASY female coloration and health that likely influenced reproductive success.

We found that variation in hatch dates within SY females was associated with plumage coloration; darker brown (more-ornamented) females laid eggs earlier in the season. This finding contradicts those in barn owls *Tyto alba*, in which darker females bred later in the season than lighter females (Roulin et al. 2001), but corroborates findings in barn swallows *Hirundo erythrogaster*, in which females with darker melanin coloration bred earlier (Safran and McGraw 2004). Moreover, darker SY females fledged more offspring and those that hatched eggs earlier appeared to benefit via increased nestling body condition, but they also laid smaller eggs, suggesting that they paid costs associated with either breeding earlier or producing darker plumage. McCarty and Secord (2000) also found that brighter SY females laid heavier eggs. Less-ornamented SY females may have avoided these costs by reducing melanin in their feathers or by laying eggs later in the season allowing them to produce heavier eggs, but which also resulted in poorer nestling condition at fledging. However, other studies of tree swallows have shown that egg size is

associated with improved offspring immunity and growth rates (Whittingham et al. 2007).

In cavity-breeding birds, competitive ability likely influences hatch date and thus can benefit reproductive success indirectly. Among birds that display melanin-based traits, those with darker (less bright) plumage generally have greater melanin concentrations in feathers (McGraw et al. 2004, 2005). Melanin pigmentation in passerines can be influenced by circulating hormones, such as testosterone (Gonzalez et al. 2001, McGraw 2006) and may act as a signal of competitive ability (Senar 1999, McGraw et al. 2002, 2003). Thus, darker SY female tree swallows may have higher levels of plumage melanin and testosterone, enabling them to out-compete brighter (less ornamented) SY females for access to nest sites. However, without having measured testosterone concentrations we can only suggest this as one possible mechanism.

Among ASY females, we found a positive relationship between ornamentation and egg mass, which corroborates Bitton et al.'s (2008) findings. Nevertheless, more-ornamented ASY females (brighter, greater blue chroma, and shorter-wavelength hues) produced offspring with lower body condition at fledging. We also failed to find a relationship between plumage coloration and number of nestlings fledged, which contradicts the findings of Bitton et al. (2008) who found that female tree swallows with bluer feathers fledged more offspring. The negative relationships between ASY ornamentation and nestling condition may be indirect and result from the negative relationship between ornamentation and cell-mediated immunity. Classical sexual selection theory predicts positive covariation between ornamentation and immunity (Andersson 1994). Indeed, in male birds, a positive relationship between structurally-based plumage coloration and cell-mediated immunity has been found (Griggio et al. 2010b), but no relationship has been defined for females (Parn et al. 2005). Additionally, we found a negative relationship between ornamentation in ASY females and hematocrit values; an indicator of reduced oxygen-carrying capacity and infection (Cooper 1975, Harrison and Harrison 1986, Brown 1996).

Iridescent structurally-based plumage coloration is likely costly to produce; stress during feather development (Doucet et al. 2006), experimental food deprivation (McGraw et al. 2002), and disease (Hill et al. 2005) reduce iridescent coloration. Moreover, iridescent plumage may be more costly to maintain than other plumage types; iridescent plumage requires more preening to sustain the hydrophobicity of feathers compared to non-iridescent plumage (Eliason and Shawkey 2011). Therefore, more-ornamented iridescent plumage could incur maintenance costs that detract time from other activities, such as foraging (Walther and Clayton 2005). In our study, the most-ornamented ASY females produced nestlings in poorer body condition, indicating these females may experience health-related costs associated with producing iridescent plumage or they invest more energy in feather maintenance and allocate less energy towards parental care. While we did not test this, one possible benefit of having increased ornamentation could arise from assortative mating with respect to plumage coloration in tree swallows (Bitton et al. 2008). More-ornamented males are typically in better

condition (Andersson 1994, Bitton and Dawson 2008) and sire more offspring (Bitton et al. 2007). Therefore, assortative mating for iridescent coloration could increase female fitness through producing more attractive sons (sexy son hypothesis; Weatherhead and Robertson 1979).

Alternatively, relationships within ASY female coloration, health, and immunity could be driven by female age. Iridescent ornamentation increases with age in adult females (Bitton et al. 2008) and tree swallows experience a decrease in cell-mediated immune response as they become older (Hausmann et al. 2005). This trend in immune function is likely due to an involution of the thymus with age; the thymus produces T cells and is primarily responsible for cell-mediated immunity (Davison 2008). Senescence has also been shown to lead to a higher parasite load (Moller and de Lope 1999), possibly as a result of a lower cell-mediated immunity, which could decrease hematocrit levels. Senescence of the immune system, however, does not necessarily explain lower hematocrit levels in the more ornamented older females. In fact, studies tend to find an increase in hematocrit levels from nestlings to adults due to an increase in RBC production; however, when comparing among adults no relationship between hematocrit and age is expected (reviewed by Fair et al. 2007) with the exception of the positive relationship found by Norte et al. (2009). Therefore, it is unlikely that the hematocrit levels we found are a result of age and indicate that more-ornamented females experience a health-related cost. Indeed, reproductive success in tree swallows has been shown to improve until 4 yr of age and then decline (Robertson and Rendell 2001). Unfortunately, we were unable to age adults past their second year of breeding due to low return rates in our population.

Conclusion

Our study was correlational in nature and should be interpreted conservatively; however, it does suggest a clear need for experimental research focusing on the signaling function of age-specific plumage in females. The relationships we found between plumage ornamentation, condition, and reproductive parameters within age-classes imply that there could be costs, especially for females experiencing competition for limited breeding resources, associated with producing age-related plumage. Future research should focus on the signaling function of plumage within the SY age class and the physiological costs of maintaining iridescent structurally-based plumage coloration and the potential trade-offs it could elicit. Furthermore, it would be interesting to test whether the change in coloration as females age from SY to ASY is associated with changes in reproductive investment.

Acknowledgements – We thank Nathan Soley, Maggie Yokley, David Hamilton, Maggie Carey, Kathryn Stainback, Molly Watson, Katherine Bakewell, Edward Burrell, and Rose Ennis for help with data collection and processing. We thank Guishan Huo for microscopy training. Appalachian State Univ. Office of Student Research provided financial support and the Valle Crucis Conference Center and the Taylor family kindly allowed us access to their land.

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