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Blood Parasite Infection and Plumage Elaboration of Migratory and Resident Populations of the Common Yellowthroat

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BLOOD PARASITE INFECTION AND PLUMAGE ELABORATION IN MIGRATORY AND RESIDENT POPULATIONS OF THE COMMON YELLOWTHROAT

by

Rebecca L Schneider

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ABSTRACT

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In birds, blood parasite diversity varies with latitude, seasonal changes, and habitat type. As a consequence, migratory populations of birds have greater exposure to parasites during their annual cycle than resident populations. Parasite infections may negatively affect the health of individuals which could, in turn, affect the development of elaborate plumage ornaments. We compared migratory (Wisconsin) and resident (Florida) populations of common yellowthroats (*Geothlypis trichas*) to test whether migratory birds in Wisconsin have more prevalent, intense and diverse infections of blood parasites and subsequently less elaborate ornamentation than their resident counterparts in Florida. As predicted, we found higher prevalence, intensity and diversity of blood parasites in Wisconsin than Florida birds. In terms of ornamentation, we found that Wisconsin males had brighter plumage in the yellow and ultraviolet spectra. These results demonstrate that blood parasite infection and plumage elaboration both differ between conspecific populations, possibly because of differences in migratory behavior.

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INTRODUCTION

Seasonal migration is a costly behavior that can increase exposure to harmful parasites, thus affecting the health of migratory individuals (Altizer et al. 2011). Avian blood parasites (Hematozoans) commonly infect most populations of birds (Valkiūnas 2005), but the extent of infection varies among species (Ricklefs 1992) and in relation to migratory behavior (Arriero and Møller 2008). Neotropical migrants are particularly susceptible to Hematozoan infection during their annual cycle as they move between temperate breeding areas, stopover or staging sites, and wintering areas (Hellgren et al. 2013). The prevalence of Hematozoan parasites is greater at lower latitudes (Greiner et al. 1975, Scheuerlein and Ricklefs 2004) and infections can be acquired on either the breeding or wintering grounds (Waldenström et al. 2002, Ricklefs et al. 2005). Thus, migratory birds are expected to have a greater exposure to parasites than resident conspecifics. Among migrants, those that are infected with parasites tend to be in poorer body condition than uninfected individuals (Dawson and Bortolotti 2000, Garvin et al. 2006). As a consequence, blood parasites might also have a negative impact on the ability to defend territories and provide parental care (Hakkarainen et al. 1998), or invest in mating effort, such as displays or ornaments (Hamilton and Zuk 1982, Sheldon and Verhulst 1996).

Elaborate plumage ornamentation in birds is thought to honestly signal the health and vitality of the individual (Hill 1991, Hill and McGraw 2006), and this relationship could be influenced by migratory behavior (Fitzpatrick 1994) if migrants are more heavily parasitized than residents. In this case, migrants may need to divert more resources away from plumage development and toward immunological responses to parasites and the metabolic demands of migration (Møller and Erritzøe 1998, Norris and Evans 2000, Hegemann et al. 2012). As a result, migrants may be expected to have less elaborate plumage ornaments than residents. Most studies of the relationship between parasites or plumage ornamentation and migration have compared

migrants and residents of different species (e.g. Deviche et al. 2001, Waldenström et al. 2002, Owen and Moore 2006, Carbo-Ramirez and Zuria 2015). A more direct approach is to compare migratory and resident populations of the same species, thus controlling for phylogeny.

In this study I examined a migratory (Wisconsin) and resident (Florida) population of common yellowthroats (*Geothlypis trichas*) to determine how they differed in parasitism and ornamentation. This species of wood warbler is widespread throughout North America. The male common yellowthroat displays two prominent plumage ornaments: a black mask and a yellow bib. In Wisconsin, females prefer to mate with males that have larger black masks (Thusius et al. 2001), and mask size is related to immune activity (Garvin et al. 2008), immune gene variation (Dunn et al. 2012) and male dominance (Tarof et al. 2005). Assuming that migratory populations are at greater risk of blood parasite infection than resident populations, we predicted that Wisconsin migrants will have higher prevalence, intensity and diversity of infection than resident Florida birds. We also predicted that if infection with blood parasites is correlated with reduced elaborate ornamentation, then migrant Wisconsin individuals will have duller or smaller plumage ornaments than Florida residents, and that highly infected individuals within a population will have less elaborate ornaments than those with little or no infection.

METHODS

Study species and field methods

Common yellowthroats breed in temperate and subtropical latitudes throughout North America. Temperate breeding populations are seasonally migratory, while populations that occupy more southern latitudes remain resident year-round. Common yellowthroats can occupy diverse habitats, but are generally found in wetland areas (DeGraaf and Rappole 1995). Both

migrant and resident common yellowthroats breed in the early spring and summer and molt in the late summer and early fall (Pyle et al. 1997).

We sampled two populations of common yellowthroats: a migratory population in Saukville, Wisconsin, USA (43°23'13.6"N 88°01'22.3"W), and a resident population in Placid Lakes, Florida, USA (27°14'06.7"N 81°24'20.5"W). The Wisconsin field site is a bog, which is predominantly wet with dense deciduous vegetation including marsh grasses and small trees. The Florida field site is a sandhill-scrub habitat that is relatively open, dominated by palmetto (*Sabal* spp. and *Serenoa* spp.), and contains several shallow ephemeral ponds. In Florida, the habitat is dry during most of the breeding season, whereas the Wisconsin bog becomes wetter throughout the breeding season. We sampled males from the Wisconsin population from May through June and in late August in 2006, 2012-2014 (n = 263), and in Florida during June 2007 and 2012, and May 2014 (n = 76).

We captured individuals using mist nets and playback recordings of male song, and measured the wing, tail and tarsus length, and body mass. A small (10-50 µL) blood sample was collected from the brachial vein in heparinized capillary tubes; a portion was used to make a blood smear and the remainder was stored in Queen's lysis buffer (Seutin et al. 1991) for DNA analysis. After processing, birds were released and resumed normal behavior within a few minutes.

Detection of blood parasite infection

We extracted DNA from whole blood using a GeneJET Whole Blood Genomic DNA Purification Mini Kit (ThermoFisher Scientific, Waltham, MA). The presence of blood parasites in each bird was determined using the nested-PCR approach of Waldenström et al. (2004)

(Wisconsin *n* = 140, Florida *n* = 124). The initial set of primers, HaemNF1 and HaemNR3, amplify a 580-bp region of cytochrome b in both *Plasmodium* and *Haemoproteus* genera parasites. In a total volume of 25 µL, PCRs consisted of 1.6 µM each of forward and reverse primers, 1.5 mM of $MgCl_2$, 5 µL of 5x Green GoTaq Flexi buffer, 200 µM dNTPs, 1 U GoTaq DNA polymerase, 13.8 µL of water, and 25-200 ng of genomic DNA. PCR amplifications started with an initial denaturing step at 94° C for 3 min followed by 20 cycles of 30 s at 94° C, 30 s at 50° C and 45 s at 72° C.

The second PCR uses internal primer sites nested within the first PCR product to identify the specific parasite genera (*Plasmodium* or *Haemoproteus*) in the samples. Here, we used 1 µL of the product from the initial PCR as template, along with the same concentrations of PCR reagents and under the PCR conditions above with 35 cycles, and the nested-PCR primers HaemF and HaemR2. To confirm a positive infection, we visualized the products of the second PCR on a 2% agarose gel run at 100 v for 40 min for presence of a band at approximately 500 bp. Negative results (i.e. no band) were repeated two additional times. Positive samples were sequenced at the University of Chicago Cancer Research Center DNA Sequencing Facility. We aligned the forward and reverse sequences using Geneious 7.1.9 software (Biomatters Ltd, http://www.geneious.com/, Kearse et al. 2012) and identified the parasite genera and lineage using BLAST (NCBI) and cross-referencing to Pagenkopp et al. (2008).

To determine intensity of infection for each individual, we analyzed blood smears using a compound light microscope. Slides were fixed with methanol then prepared by staining with a Dip Quick Stain Kit (Jorgensen Laboratories, Inc., Loveland, CO, USA) and examined at 1000 x magnification. We recorded the number of visibly infected red blood cells out of a total of 4000 red blood cells in individuals from Wisconsin (*n* = 120) and Florida (*n* = 75).

Plumage measurements

To measure plumage ornaments, we held each individual in a standardized position against a 1-cm^2 black and white grid and recorded images of the bib and the left and right profiles of the facial mask. We captured still images from the video recordings using video editing software (iMovie '11 for Mac OS X 10.6, Apple, Inc.) and imported them into an image analysis software (ImageJ 1.49v, http://imagej.nih.gov/ij). Setting each picture to scale based on the grid background, we measured the mask in two still images from each side by tracing the ornament. Mask size (mm^2) was recorded as the sum of the left and right after averaging the two measurements, whereas we measured bib size as the total yellow area on the breast in one image. Repeatability between observers for these measurements was 0.99 and 0.87 for mask and bib size, respectively.

We assessed plumage brightness, hue, and saturation by plucking five yellow bib feathers from the center of the bib. We overlapped the individual feathers and mounted them on a black matte background then used a spectrophotometer (USB2000, Ocean Optics, Dunedin, Florida) to measure the following: feather reflectance (R), estimated mean brightness (average reflectance) in the UV (320-400 nm) and yellow range (550-625 nm), hue (wavelength of maximum reflectance between 320 and 700 nm), and saturation (sum of reflectance values in the yellow range divided by the total brightness; Dunn et al. 2008, 2010). We also estimated carotenoid chroma (C_{car}) which represents the yellow saturation due to the concentration of carotenoids in the feather (Andersson and Prager 2006). C_{car} was calculated as $(R_{700nm}-R_{450})/R_{700nm}$ (see Freeman-Gallant et al. 2010).

Statistical analyses

We conducted all analyses in JMP 11 PRO (SAS Institute, Inc. Cary, NC, USA). For comparisons between WI and FL of plumage ornaments and parasitic infection, we used non-parametric ANOVA of each variable by population. To analyze the relationship between measures of parasite infection and plumage, we used a general linear model that included population (WI or FL), date (Julian), year, and body size (mass, tarsus) as predictors.

RESULTS

We found a difference between populations in both blood parasitism and plumage ornamentation. In Florida (FL), fewer individuals were infected with *Plasmodium* (15% of males; 11/71) than in Wisconsin (WI; 84% of males, 93/111; $\chi^2 = 82.5$, df = 1, $P < 0.0001$, Fig. 1a). Infections were also less intense in FL than in WI, as the proportion of infected cells was lower in FL (0.0006%, $n = 41$ slides) than WI individuals (0.006%, $n = 80$ slides; Kruskal-Wallis test, Z = 5.41, df = 1,120, $P < 0.0001$, Fig. 1b). There were only two lineages of *Plasmodium* in FL (4b and 6a, GenBank Accession EU328173 and EU328172, Pagenkopp et al. 2008; $n = 6$ infections successfully sequenced), compared with four lineages in WI (4b, 6a, 6b, and 9a, GenBank Accession EU328173-EU328170, Pagenkopp et al. 2008; $n = 56$ infections successfully sequenced). Sample sizes of sequenced results vary because we were not able to identify the lineage for all samples. Although the Waldenström primers are able to amplify *Haematoproteus*, we did not amplify any lineages in that genus.

Males in FL had larger black facial masks and smaller yellow bibs than birds in WI (both $P < 0.0001$, Table 1, Fig. 2a and b). Since FL birds were also larger than WI birds, we controlled for body size by including mass and tarsus as predictors in the models comparing plumage

ornaments between populations (Table 2). The yellow bibs of FL males were duller (lower reflectance in both the ultraviolet and yellow portions of the spectrum) than WI males (Table 3, Fig. 2c and d). Other indices of coloration (saturation, hue, and carotenoid chroma) differed between populations but did not account for a substantial amount of the total variation (Table 3).

The presence of *Plasmodium* infection was negatively correlated with UV brightness and yellow brightness among males in the FL population, but not in WI (UV brightness $F_{3, 67} = 3.9$, $P = 0.01$; yellow brightness $F_{3, 67} = 6.01$, $P = 0.001$; Fig. 3a,b). In contrast, the intensity of infection was negatively correlated with UV brightness and yellow brightness among males in WI, but not in FL (UV brightness $F_{3,73} = 4.8$, $P = 0.004$; yellow brightness $F_{3,73} = 2.65$, $P = 0.05$; Fig. 4a,b). Overall, WI males had brighter bibs than FL males in both the UV and yellow range, in analyses that controlled for infection intensity or the presence of infection and male size (Table 4). Blood parasite infection (presence/absence) was not associated with the size of ornaments (mask or bib) in either population (Table 5), nor was it associated with other measures of bib coloration (saturation, hue, and carotenoid chroma, Table 6). In FL, *Plasmodium* lineage 6a was associated with lower saturation of bib feathers ($F_{1,4} = 13.08$, $P = 0.02$). In WI, no lineages of blood parasite were related to plumage ornamentation.

DISCUSSION

Blood parasite infections are expected to be greater in migratory than resident populations of birds and may impact the production or expression of colorful plumage ornaments. As predicted, we found that individuals in a migratory population (Wisconsin) of common yellowthroats had higher prevalence, intensity, and diversity of infection with blood parasites than individuals in a resident population (Florida). In each population we also found

support for our prediction that highly infected individuals would have duller plumage than those with little or no infection. It is important to note that in Florida, where individuals had low levels of parasitism, duller plumage was related to the presence of infection (Fig. 3), whereas in Wisconsin, where most individuals were infected, duller plumage was related to the intensity of infection (Fig. 4). Between populations, contrary to our prediction, we found that males in Wisconsin had brighter bibs than males in Florida despite a higher infection rate with blood parasites. These results suggest that increased parasitism is associated with migratory behavior, and that within populations parasitism has a negative effect on plumage traits.

Parasitism and migration

Migrants are exposed to multiple habitat types across latitudes throughout their annual cycle, and thus may accumulate more parasites than residents (Figuerola and Green 2000). Ecological factors associated with parasite abundance, such as lowlands and freshwater, are general predictors of infection (Piersma 1997). In addition, parasite burden during the overwintering period increases, especially at lower latitudes (Clark et al. 2016). Common yellowthroat range maps show that migrants occupy lower latitudes during the overwintering period than residents, and thus migrants could experience this burden more intensely. In support of the hypothesis that migration increases exposure to parasites, we found that males in the migratory Wisconsin population were more commonly infected with blood parasites and had more lineages of *Plasmodium* than resident males in Florida. This is consistent with previous work that found greater parasite species richness in migratory than resident populations of birds and butterflies (Altzier et al. 2000, Koprivnikar and Leung 2014).

If parasites are more common in particular geographic areas, seasonal migrants may be

able to avoid certain habitats, and thus reduce the risk of infection during the annual cycle (Piersma 1997). Parasite prevalence shows some geographic structure across the range of common yellowthroats, and the *Plasmodium* lineages identified in both our populations were consistent with the structure of geographic diversity reported by Pagenkopp et al. (2008). The distribution of these parasite lineages could be explained by variation in vector abundance, however (Pagenkopp et al. 2008). Since the southwestern United States is very dry, for example, it does not provide good habitat for mosquitoes, and indeed the prevalence of haemosportidians in common yellowthroats is very low in this region (19.7% of birds infected compared to 78.5% in the Midwest; Pagenkopp et al. 2008). Dry, vector-poor regions that still provide a hospitable climate year-round for birds, then, are ideal for resident populations. The common yellowthroat population we studied in Florida occupies a comparably drier habitat (ephemeral bays) than Wisconsin during the breeding season. Although there is more rainfall during the breeding season at our study site in Florida (25" on average) than our site in Wisconsin (12" on average), there is less standing water at the Florida site, which likely reduces the abundance of mosquitoes. Thus, fewer vectors (mosquitoes) may be one of the main reasons we found less parasitism in Florida compared to Wisconsin.

There inevitably exists a significant trade-off between migration and immune capacity (Piersma 1997) because migration is energetically costly and can reduce immune function (Norris and Evans 2000). In addition to the actual energetic cost of long distance migration, immunomodulations, the changes in immune function that occur before birds undergo migration such as suppression of cell-mediated immunity, not only increase susceptibility to pathogens but may also lead to relapse of previous infections (Valkiūnas 1991, Altizer et al. 2011). Thus, due to the physiological cost of migration, the immune system of migratory birds may not be as

effective at combating parasites as residents (Buehler and Piersma 2008, Buehler et al. 2010), resulting in more intense infections among migrants. Møller (1998) found that among pairs of tropical and non-tropical species of birds, those in the tropics had better immune function. In common yellowthroats, lower parasite infection of resident Florida birds is consistent with the idea that residents may have better immune function than Wisconsin migrants. However, it is possible that migrants have evolved an optimized immune function in response to increased parasitic exposure (Møller and Erritzøe 1998) and therefore do not experience adverse effects of a high parasitic load. Moreover, since avian blood parasites are widely distributed, are more common and more diverse at lower than higher latitudes, and are more prevalent in wetter environments (Greiner et al. 1975, White et al. 1978, Valkiūnas 2005, Clark et al. 2014, Von Rönn et al. 2015), seasonal migratory movements over a wide geographical range should subject migrants to higher parasitism than residents regardless of the immunological cost of migration. Alternatively, recent studies have proposed that regular long-distance movements by migratory animals allow hosts to escape infected habitats and could lower the virulence of the dispersed pathogens (Altizer et al. 2011, Hall et al. 2014), while others have shown that populations that have lost migratory behavior are at increased risk for infection with pathogens (Satterfield et al. 2014).

Ornamentation and migration

It is well established that ornaments, such as colorful feathers, play an important role in attracting mates (Andersson 1994). The degree to which an individual can express an elaborate and often costly plumage ornament is dependent on physiological and environmental factors (Zuk et al. 1990, Hill 2002, Norris et al. 2004). Migratory behavior may therefore affect the

expression of ornaments due to the physiological changes needed within an individual before and after migration, such as premigratory fattening or change in gonad size, as well as the habitat quality during molt. If birds molt into their breeding plumage during the non-breeding season, then high quality wintering habitats can have an important influence on brighter breeding plumage (Reudink et al. 2009). However, common yellowthroats breeding in Wisconsin molt only once a year, at the end of the breeding season on the breeding grounds (Pyle 1997), and thus, the quality of the breeding habitat is more important to the expression of their plumage. In contrast, resident birds, such as those in Florida, do not experience the same migration-associated physiological costs and their ability to express ornaments is entirely dependent on the quality of a single year-round habitat. In our study, male common yellowthroats in the migratory Wisconsin population had brighter plumage in both the UV and yellow portions of the spectrum than males in the resident Florida population. This does not support our prediction that the cost of migrating and harboring more parasites reduces the expression of plumage ornaments among migrants compared to residents. Perhaps this is not surprising as parasites are not the exclusive drivers of ornament expression; dietary resources, quality of habitat and timing of molt can also make an important contribution to the expression of ornaments (Hill et al. 2002, Norris et al. 2004, Serra et al. 2007, Reudink et al. 2009). In addition, selection on plumage coloration may differ between reproductively isolated populations (Ruegg 2008).

Parasitism and ornamentation

Hamilton and Zuk (1982) proposed that within a population the expression of secondary sexual ornaments is determined by the individual's degree of parasitism, specifically that brighter plumage is associated with fewer parasites. While their hypothesis has been repeatedly

tested in both avian and non-avian systems (Piersma et al. 2001, Miliniski and Bakker 1990), the results have been equivocal with some studies providing support (Read 1987, von Schantz et al. 1996, Scheuerlein and Ricklefs 2004) while others do not (Read and Weary 1990, Hamilton and Poulin 1997, Martin and Johnsen 2007). We found that within populations of both migratory and resident common yellowthroats, males with more blood parasites had duller plumage, supporting one prediction of the Hamilton and Zuk hypothesis. Between populations, we found that the more highly parasitized migrants in Wisconsin have brighter feathers than the less parasitized residents in Florida. In fact, the mean brightness for the most intensely infected males in Wisconsin was brighter than the mean for uninfected males in Florida (Fig. 4). This seems contrary to the prediction that individuals heavily infected with parasites cannot express bright plumage signals, however it is consistent with Hamilton and Zuk's prediction that species with high parasitic pressure should evolve more conspicuous ornaments than those without.

Although there have been studies that have examined parasitism in both migratory and resident birds, most have focused on comparisons between closely related species (Møller 1990, Deviche et al. 2001, Carbó-Ramírez and Zuria 2015). However, a more conservative and direct approach is to compare different populations within a species. For intraspecific comparisons, Zuk (1991) predicted that residents should demonstrate a relationship between parasitism and brightness more strongly than migrants due to sustained coevolution between pathogens and hosts at a single site. Assuming that populations residing in the tropics face high parasitic pressure, those birds will evolve showier ornaments than conspecific migrants. This is due to strong selective pressure to display an honest plumage ornament indicative of tolerance to parasites (Hamilton and Zuk 1982), which is particularly intense for residents which coexist with the same suite of parasitic fauna throughout the year. Our results show that the assumption made

by Zuk (1991) is not accurate, and that in fact migrants may face more parasitic pressure than residents of the same species. The predicted subsequent positive effect on ornament expression is still upheld, however. Since Wisconsin migrant common yellowthroats have greater abundance and diversity of pathogens than Florida residents, selection favoring an honest signals of heritable resistance may be stronger resulting in more elaborate ornament expression. Studies that contest the strength of the evolutionary relationship between parasitism and ornamentation emphasize the fact that there is limited support for the Hamilton and Zuk (1982) hypothesis in intraspecific comparisons (Hamilton and Poulin 1997), but our results show support for both predictions on a within-species population level.

In this study, we found differences in parasitic infection and ornament production in two populations of the common yellowthroat. Overall, infection with blood parasites was positively related to migratory behavior, and negatively related to plumage brightness. Migrants, however, had brighter plumage while still harboring more parasites than residents. Future work should focus on testing additional conspecific migratory and resident populations to determine if these patterns are due to migratory behavior or other ecological effects, such as habitat, which can differ between temperate and subtropical locations and influence the rate of parasite transmission.

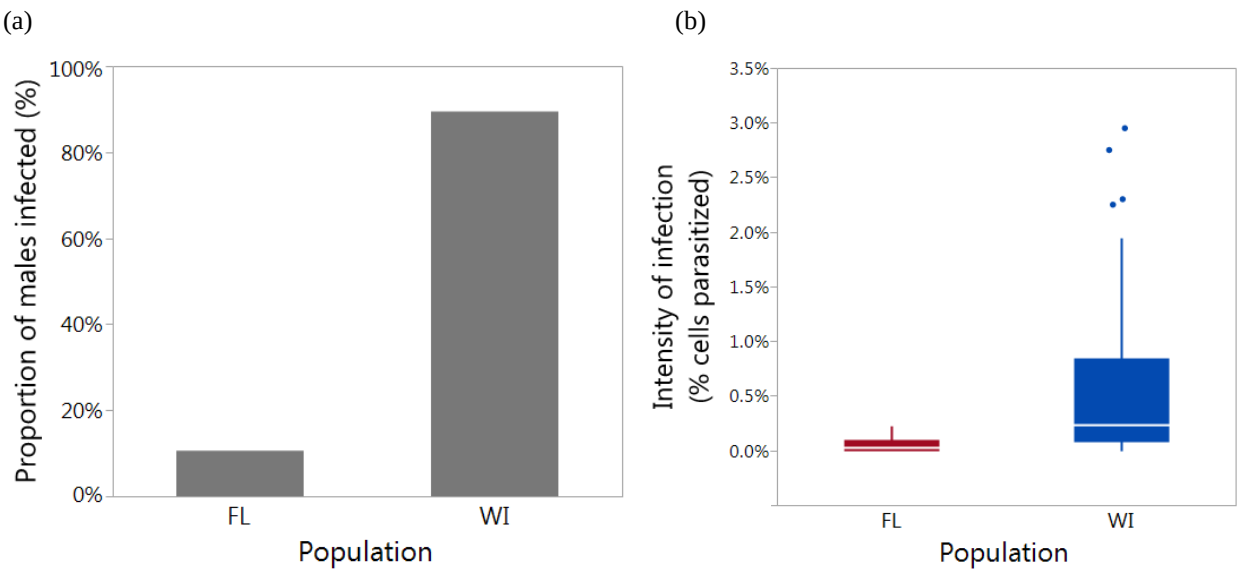

Figure 1. Differences between resident (FL) and migratory (WI) populations in presence of infection as defined by proportion of males infected (a) and intensity of infection (b). Presence of infection was based on PCR and intensity of infection was based on blood smears. Box plots in b show the interquartile range and median; vertical lines indicate the minimum and maximum values, or, the outermost data points that fall within 1.5 times the interquartile range with points indicating outliers. For both response variables, presence of infection and intensity of infection, individuals in WI had significantly greater infections than individuals in FL ($P < 0.0001$).

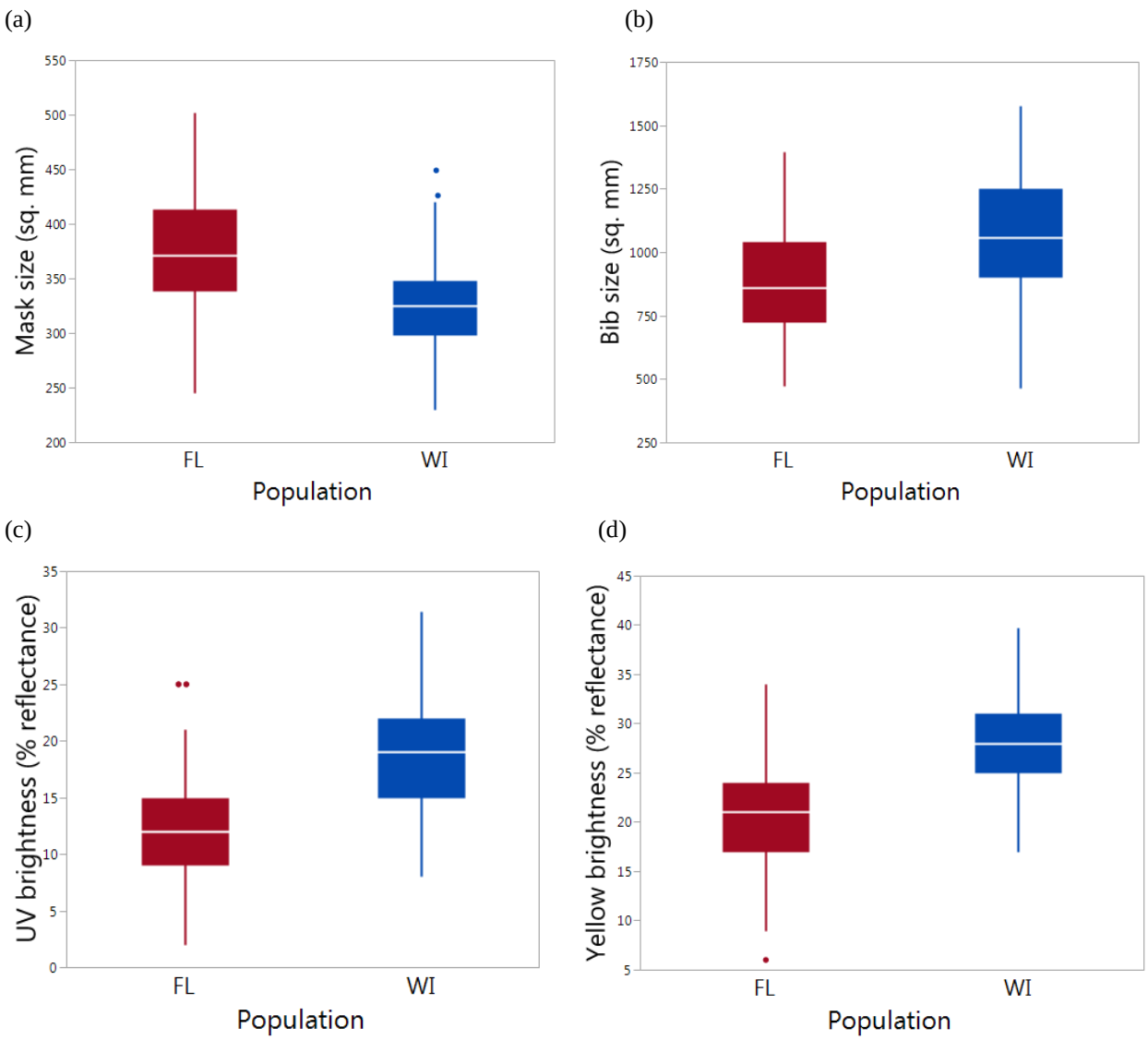

Figure 2. Difference in ornament elaboration between resident (FL) and migratory (WI) populations. Box plots show the interquartile range and median; vertical lines indicate the minimum and maximum values, or, the outermost data points that fall within 1.5 times the interquartile range with points indicating outliers. Individuals in the FL population had larger black masks (a) and smaller yellow bibs (b) than individuals in the WI population ($P < 0.0001$; Table 1). Yellow bibs were brighter in both the UV (c) and yellow spectra (d) in WI than in the FL population ($P < 0.0001$; Table 1).

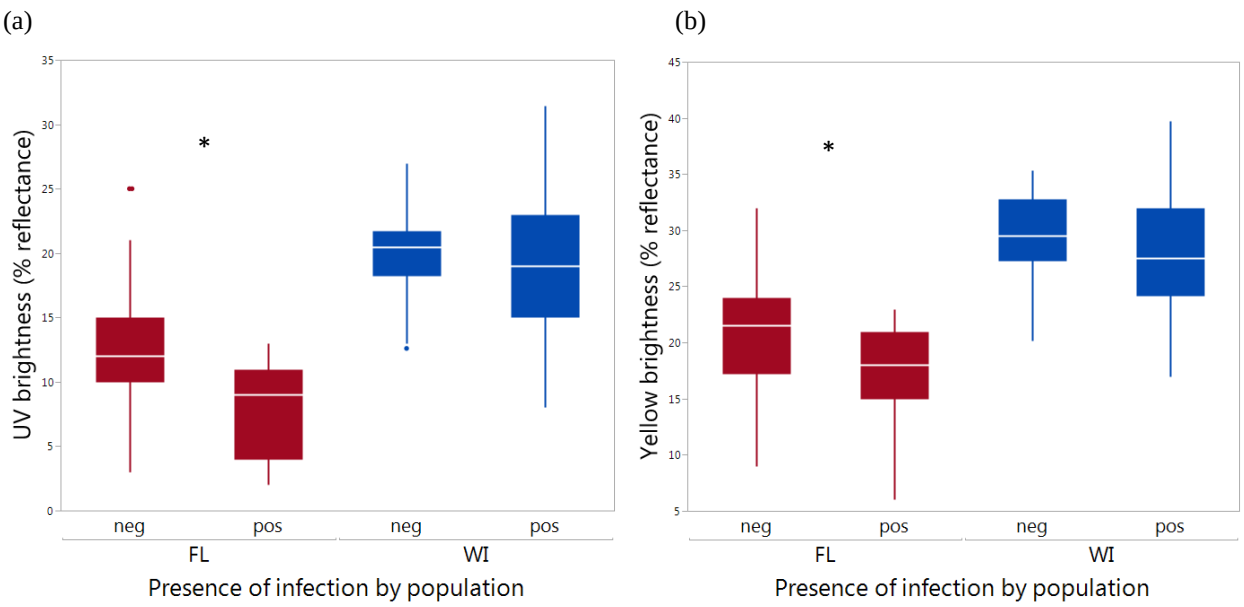

Figure 3: The relationship between blood parasite infection and bib brightness in the UV (a) and yellow (b) spectra by population (WI or FL). Presence of infection (positive/negative) was based on PCR results. In FL infected males had duller bibs in the UV (P = 0.01) and yellow (P = 0.001) portions of the spectrum than birds that were not infected. The presence of infection was not related to plumage brightness for males in WI. Shown are univariate relationships; see Tables 3 and 4 for results from the full model. Box plots show the interquartile range and median; vertical lines indicate the minimum and maximum values, or, the outermost data points that fall within 1.5 times the interquartile range with points indicating outliers.

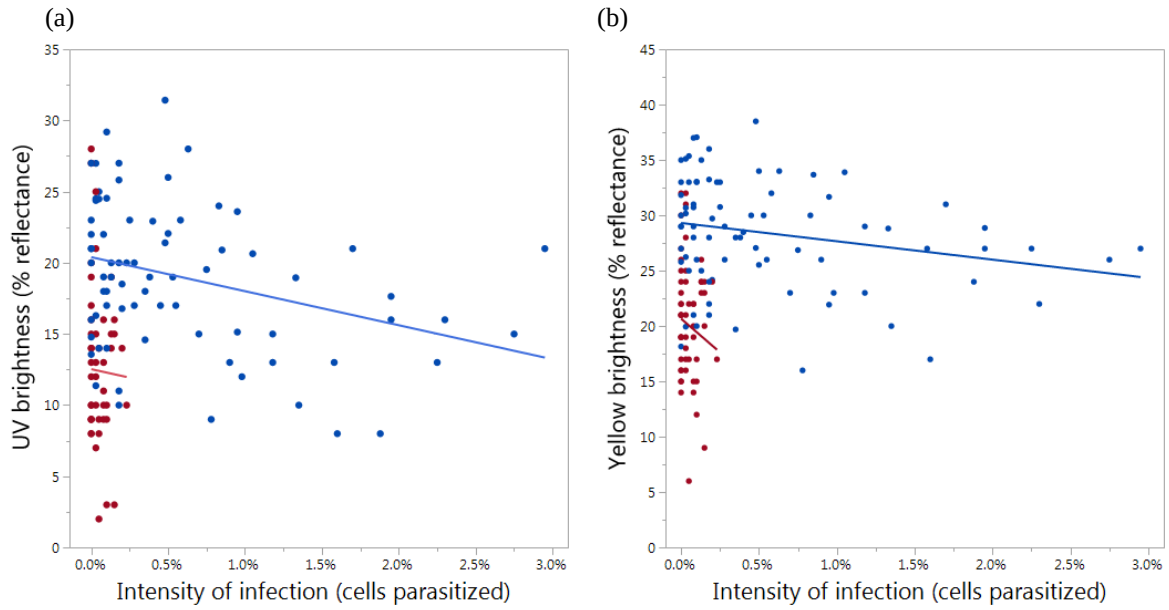

Figure 4: The relationship between infection intensity and bib brightness in the UV (a) and yellow (b) spectra by population (WI or FL). Infection intensity was based on blood smear slides and represented as a proportion of observed intracellular infections per 4000 cells. The FL population is shown in red and the WI population is shown in blue. Bib brightness is represented by percent reflectance in each color range. In WI, higher infection intensity was correlated with duller plumage in the UV spectrum ($P = 0.004$) and in the yellow spectrum ($P = 0.05$). In FL, infection intensity was not related to bib coloration.

Table 1: Plumage ornament size and bib coloration for males in Florida and Wisconsin populations. Model effects include population, year, Julian date, tarsus, and mass.

	Florida					Wisconsin				
	Mean	SE	Lower CI	Upper CI	*n*	Mean	SE	Lower CI	Upper CI	*n*
Mask size	373.75	5.68	361.59	383.96	68	322.76	3.21	316.82	329.46	175
Bib size	884.39	36.28	846.19	989.15	67	1074.69	20.41	1021.36	1101.78	170
UV brightness	12.07	0.60	11.30	13.67	74	18.85	0.35	18.01	19.38	192
Yellow brightness	20.66	0.61	19.65	22.06	74	27.91	0.35	27.14	28.53	192
Hue	612.55	1.19	612.36	617.06	74	610.59	0.69	608.43	611.15	192
Saturation	0.29	<0.01	0.28	0.29	74	0.28	<0.01	0.28	0.28	192
Carotenoid chroma	0.83	0.01	0.83	0.86	74	0.82	0.01	0.81	0.83	192

Table 2: Measures of ornament size in relation to study population (WI/FL), year, date of capture (Julian date), body mass and tarsus length. Least-squares analysis full model.

	Mask size				Bib size			
	Slope	SE	*t*	*P*	Slope	SE	*t*	*P*
Intercept	-7817.6	2146.90	3.6	0.0003	-57696.3	14737.15	-3.92	0.0001
WI or FL	24.8	3.53	7.0	<0.0001	-72.0	22.75	3.2	0.002
Year	4.0	1.07	3.7	0.0002	28.9	7.36	3.9	0.0001
Julian date	-0.4	0.13	3.0	0.003	0.5	0.82	0.6	0.58
Mass	11.2	5.25	2.1	0.03	-14.0	33.27	0.4	0.67
Tarsus	0.2	4.59	0.0	0.97	35.5	29.41	1.2	0.23
Model R^2, F, P	$R^2 = 0.32$, $F_{5,237} = 22.1$, $P < 0.0001$				$R^2 = 0.17$, $F_{5,231} = 9.6$, $P < 0.0001$			

Table 3: Measures of plumage coloration in relation to population (WI/ FL), year, and date of capture (Julian date). Least-squares analysis full model.

	UV brightness				Yellow brightness			
	Slope	SE	*t*	*P*	Slope	SE	*t*	*P*
Intercept	-261.4	282.39	0.9	0.36	-243.0	287.24	0.9	0.40
WI or FL	-3.2	0.36	8.7	<0.0001	-3.5	0.37	9.5	<0.0001
Year	0.1	0.14	1.0	0.33	0.1	0.14	0.9	0.35
Julian date	-0.001	0.01	0.1	0.93	-0.01	0.01	0.6	0.53
Model R^2, *F*, *P*	$R^2 = 0.28$, $F_{3,262} = 34.6$, $P < 0.0001$				$R^2 = 0.32$, $F_{3,262} = 40.2$, $P < 0.0001$			

	Hue				Saturation				Carotenoid chroma			
	Slope	SE	*t*	*P*	Slope	SE	*t*	*P*	Slope	SE	*t*	*P*
Intercept	-1960.8	560.24	3.5	0.001	1.2	1.12	1.0	0.30	-12.1	4.60	2.6	0.01
WI or FL	2.5	0.72	3.5	0.001	0.004	0.001	2.7	0.01	0.01	0.01	2.1	0.04
Year	1.3	0.28	4.6	<0.0001	-0.0004	0.001	0.8	0.44	0.01	0.002	2.8	0.01
Julian date	0.05	0.03	1.7	0.09	-0.000003	0.0001	0.1	0.95	0.0003	0.0002	1.4	0.17
Model R^2, *F*, *P*	$R^2 = 0.08$, $F_{3,262} = 8.1$, $P < 0.0001$				$R^2 = 0.04$, $F_{3,262} = 4.0$, $P = 0.01$				$R^2 = 0.03$, $F_{3,262} = 3.2$, $P = 0.03$			

Table 4: Plumage brightness in the UV and yellow spectrum in relation to blood parasite infection.

	UV brightness				Yellow brightness			
Predictors	Slope	SE	*t*	*P*	Slope	SE	*t*	*P*
Infection presence model								
Intercept	-505.7	298.33	1.7	0.09	-442.7	310.52	1.4	0.16
Year	0.3	0.15	1.8	0.08	0.2	0.15	1.5	0.13
Julian date	<0.01	0.02	0.6	0.53	<0.01	0.02	1.4	0.18
WI or FL	-4.1	0.51	7.9	<0.0001	-4.5	0.53	8.3	<0.0001
Infection present (0,1)	-2.3	0.98	2.4	0.02	-2.3	1.02	2.3	0.02
Model R^2, F, P	R^2 = 0.38, $F_{4,170}$ = 25.8, P <0.0001				R^2 = 0.41 $F_{4,170}$ = 29.0, P <0.0001			
Infection intensity model								
Intercept	840.1	1117.30	0.8	0.45	-1729.5	1103.91	1.6	0.12
Year	-0.4	0.55	0.7	0.46	0.9	0.55	1.6	0.11
Julian date	<0.01	0.04	0.9	0.35	0.0	0.04	0.5	0.66
WI or FL	-4.1	0.57	7.2	<0.0001	-4.8	0.56	8.7	<0.0001
% cells infected	-241.5	83.09	2.9	<0.001	-199.5	82.09	2.4	0.02
Model R^2, F, P	R^2 = 0.33, $F_{4,113}$ = 14.2, P <0.0001				R^2 = 0.43, $F_{4,113}$ = 21.4, P <0.0001			

Table 5: Ornament size in relation to blood parasite infection.

	Mask size				Bib size			
Predictors	Slope	SE	*t*	*P*	Slope	SE	*t*	*P*
Infection presence model								
Intercept	-8579.7	2381.14	3.6	<0.001	-56628.0	15559.95	3.6	<0.001
Year	4.4	1.19	3.7	<0.001	28.4	7.77	3.7	<0.001
Julian date	-0.3	0.19	1.6	0.11	0.4	1.14	0.3	0.73
Mass	13.6	6.58	2.1	0.04	11.9	40.34	0.3	0.77
Tarsus	0.6	5.26	0.1	0.91	16.0	32.51	0.5	0.62
WI or FL	27.3	5.04	5.4	<0.0001	-41.3	31.65	1.3	0.19
Infection present (0,1)	6.3	8.95	0.7	0.48	72.7	54.88	1.3	0.19
Model R^2, F, P	$R^2 = 0.37$, $F_{6,155} = 15.2$, $P < 0.0001$				$R^2 = 0.19$, $F_{6,149} = 5.7$, $P < 0.0001$			
Infection intensity model								
Intercept	6557.2	9578.93	0.7	0.50	-114598.7	57933.50	2.0	0.05
Year	-3.1	4.76	0.6	0.52	57.3	28.79	2.0	0.05
Julian date	0.4	0.34	1.1	0.28	1.7	2.05	0.8	0.41
Mass	13.7	8.49	1.6	0.11	22.3	51.35	0.4	0.67
Tarsus	-9.9	6.84	1.4	0.15	-3.7	41.39	0.1	0.93
WI or FL	36.8	5.80	6.4	<0.0001	-86.8	35.08	2.5	0.02
% cells infected	741.5	722.58	1.0	0.31	-965.8	4370.15	0.2	0.83
Model R^2, F, P	$R^2 = 0.43$, $F_{6,96} = 12.3$, $P = <0.0001$				$R^2 = 0.14$, $F_{6,96} = 2.5$, $P = 0.03$			

Table 6: Bib coloration in relation to blood parasite infection.

	Saturation				Hue				Carotenoid chroma			
Predictors	Slope	SE	*t*	*P*	Slope	SE	*t*	*P*	Slope	SE	*t*	*P*
Infection presence model												
Intercept	1.7	1.23	1.4	0.18	-1776.3	639.33	2.8	0.01	-8.8	5.14	1.7	0.09
Year	<0.01	<0.001	1.1	0.26	1.2	0.32	3.7	<0.001	<0.01	<0.001	1.9	0.06
Julian date	<0.01	<0.001	1.3	0.21	<0.01	0.04	0.8	0.42	<0.01	<0.001	1.7	0.09
WI or FL	<0.01	<0.001	2.5	0.01	3.6	1.10	3.2	<0.001	<0.01	0.01	1.8	0.07
Infection present (0,1)	<0.01	<0.001	1.2	0.22	2.6	2.11	1.2	0.22	<0.01	0.02	0.5	0.60
Model R^2, F, P	R^2 = 0.07, $F_{4,170}$ = 3.2, P = 0.01				R^2 = 0.10, $F_{4,170}$ = 4.9, P = 0.001				R^2 = 0.04, $F_{4,170}$ = 1.8, P = 0.14			
Infection intensity model												
Intercept	-15.9	4.35	3.7	<0.001	7633.8	2190.19	3.5	<0.001	-86.9	17.64	4.9	<.0001
Year	<0.01	<0.001	3.7	<0.001	-3.5	1.09	3.2	<0.001	<0.01	0.01	5.0	<.0001
Julian date	<0.01	<0.001	1.0	0.31	-0.1	0.08	1.4	0.18	<0.01	<0.001	1.5	0.15
WI or FL	<0.01	<0.001	1.7	0.09	2.5	1.11	2.2	0.03	<0.01	0.01	2.4	0.02
% cells infected	0.7	0.32	2.2	0.03	-215.5	162.87	1.3	0.19	2.7	1.31	2.1	0.04
Model R^2, F, P	R^2 = 0.17, $F_{4,113}$ = 5.6, P = 0.0004				R^2 = 0.18, $F_{4,113}$ = 6.4, P = 0.0001				R^2 = 0.24, $F_{4,113}$ = 8.7, P < 0.0001			

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