Articles

Journal of Avian Biology 48: 1263–1272, 2017
doi: 10.1111/jav.01418

Subject Editor: Staffan Bensch. Editor-in-Chief: Jan-Åke Nilsson. Accepted 29 May 2017

The relationship between blood parasites and ornamentation depends on the level of analysis in the common yellowthroat

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The Hamilton–Zuk hypothesis predicts that ornament expression is a signal of the ability of individuals to resist parasite infection. Thus, across a population (i.e. between-individuals) more ornamented individuals should have lower levels of parasitism. Numerous studies have tested this prediction and the results are mixed. One reason for these conflicting results may be that many studies have examined this relationship at the between-individual level, which may be affected by confounding factors such as selective mortality. Using within-subject centering we examined the relationship between male ornamentation and avian blood parasites at both the between- and within-individual levels. These relationships focus on differences in genetically-based resistance to parasites and the trade-off in resource allocation between parasite resistance and ornament expression within an individual, respectively. We studied male common yellowthroats *Geothlypis trichas*, which have two plumage ornaments, a yellow, carotenoid-based bib (throat and chest) and a black, melanin-based facial mask. Surprisingly, within-individuals, an increase in parasitism between years was associated with an increase in mask size and, potentially, greater concentration of carotenoids in the yellow feathers. This suggests that males may be able to tolerate an increase in parasitism and still increase ornament expression. In contrast, ornamentation was not related to parasitism at the between-individual level. Thus, our study revealed relationships between ornaments and parasitism at the within-individual level that were not present at the between-individual level. Our results highlight the importance of examining both within- and between-individual relationships as correlations between variables, such as ornaments and parasites, may depend on the level of analysis (i.e. within- or between-individuals).

Male ornaments are thought to honestly advertise male quality to females (Andersson 1994). However, there are many different genetic and physiological aspects of quality that may be selected for by females through ornaments. Hamilton and Zuk (1982) hypothesized that male ornaments may honestly advertise genetic resistance to parasites and, thus, females that mate with more ornamented males will obtain superior genes for parasite resistance for their offspring. This hypothesis assumes that ornament production and parasitic infection are both costly, and thus, within individuals there will be a trade-off between allocating resources to immune response or ornament expression. If these assumptions hold, then males in a population with lower levels of parasitism are predicted to have more elaborate ornaments than males with higher levels of parasitism (Hamilton and Zuk 1982). Numerous studies have tested this hypothesis by measuring ornamentation and parasitism across males in a population – a between-individual comparison (Martin and Johnsen 2007, Setchell et al. 2009, Vergara et al. 2012, Molnár et al. 2013, Merrill et al. 2015). However, the results of these studies have been mixed, with negative (Höglund et al. 1992, Doucet and Montgomerie 2003), positive (Korpimäki et al. 1995, Trigo and Mota 2016) and no (Dias et al. 2016) relationship reported between parasites and plumage ornaments in various species of birds. These mixed results may be a consequence of confounding factors that could either produce or obscure correlations between parasite resistance and plumage ornamentation between individuals.

Indeed, in analyses between individuals, selective mortality could result in either positive or negative relationships between parasitism and resistance alleles, depending on the virulence of parasites (Fig. 1; Westerdahl 2007). In the case of mildly deleterious parasites (Fig. 1a), we might predict a negative relationship between parasitism and resistance, because there are no individuals with high resistance and high parasitism. In the case of potentially lethal parasites (Fig. 1b), however, low resistance individuals that are highly parasitized are more likely to die before they are sampled (i.e. selective mortality). This can produce a positive relationship between resistance and parasitism because there are both low resistance individuals that are, by chance, never infected, and individuals with high resistance that can tolerate these virulent parasites. If more resistant individuals produce larger ornaments (Fig. 1c), then there should be a negative relationship between ornamentation and parasitism when parasites are mild (Fig. 1d) and a positive relationship

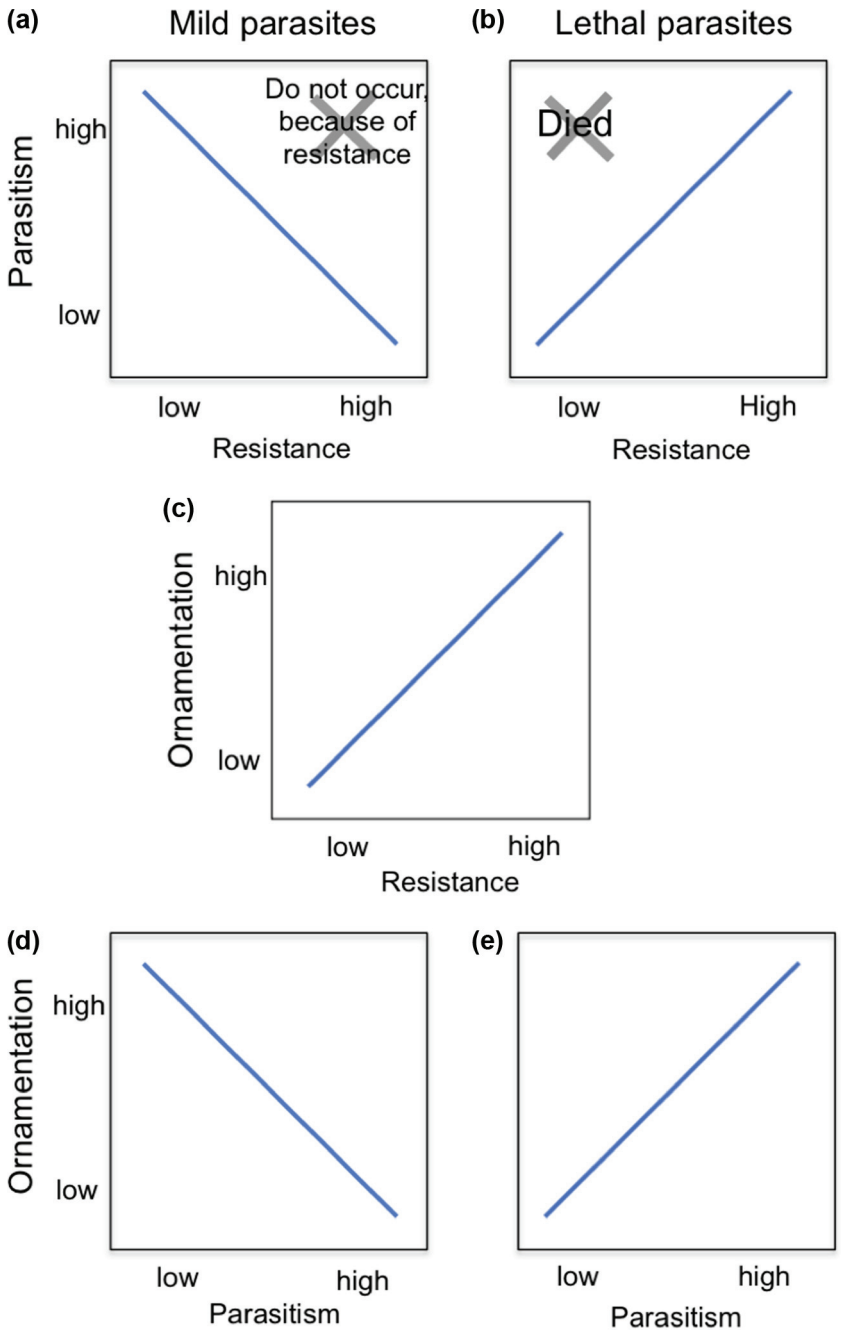

Figure 1. Schematic diagram of predicted relationships between parasitism and resistance (top row) in cases of mild (a) and lethal (b) parasites (based on Fig. 2 in Westerdahl 2007). In the case of mild parasites (a), there is a predicted negative relationship between parasitism and resistance because there are no individuals with high resistance and high parasitism. In the case of lethal parasites, there is a predicted positive relationship because low resistance individuals that are highly parasitized die before they are sampled (i.e. selective mortality). Thus, assuming that more resistant individuals produce larger ornaments (c) there is a predicted negative relationship between ornamentation and parasitism when parasites are mild (d) and a predicted positive relationship when parasites are lethal (e).

when parasites are more virulent (Fig. 1e). In this latter case (Fig. 1e), more resistant individuals can better tolerate a given parasite load, and, thus, they have relatively more resources to produce elaborate ornaments (Getty 2002).

Most studies of ornaments and parasitism have focused on between-individual analyses, but the correlation between ornaments and parasitism is influenced by variation at both the between- (population) and within-individual levels (Downs and Dochtermann 2014). Between-individual effects are driven by differences in genetically-based resistance, while within-individual effects are driven by the trade-off between resources for ornamentation and resistance to parasites. Under the Hamilton and Zuk hypothesis, the intraspecific relationship between ornaments and parasitism is predicted to be negative at both the between- and within-individual levels. However, these relationships are not necessarily the same. For example, as noted above, the between-individual relationship could be positive or negative depending on the

virulence of the parasite, even when parasitism is costly to individuals (i.e. the within-individual relationship is negative). Thus, understanding how parasites and ornamentation are related requires analyses at both the within- and between-individual levels. Although the problem of conflating levels of analysis has been widely acknowledged in evolutionary theory (Reznick et al. 2000, van de Pol and Wright 2009), it has only recently been discussed in ecoimmunology (Downs and Dochtermann 2014), and, to our knowledge, no study has partitioned within- and between-individual effects in analyses of the Hamilton and Zuk hypothesis.

In this study of common yellowthroats *Geothlypis trichas*, we analyzed both the between- and within-individual relationship between plumage ornamentation and haemosporidian parasites. Parasitism by haemosporidians negatively affects the expression of plumage ornaments in some bird species (Doucet and Montgomerie 2003, del Cerro et al. 2010), but not all (Dias et al. 2016, Purves et al. 2016). Male common yellowthroats have two plumage ornaments, a black facial mask that is melanin based and a yellow bib (throat and chest) that is carotenoid based. In our study population, females prefer social and extra-pair mates with larger black masks (Thusius et al. 2001, Tarof et al. 2005, Pedersen et al. 2006); however, there is no evidence that the size or color of the yellow bib is sexually selected (Pedersen et al. 2006). Despite the difference in selection on these two ornaments, both the mask and the bib are honest indicators of some aspects of male quality in this population. Males with both larger masks and bibs are more resistant to oxidative stress (Henschen et al. 2016). In addition, males with larger masks produce more antibodies (IgG) and are more likely to survive overwinter than small-masked males (Dunn et al. 2010). Finally, there is also evidence to suggest that males with larger masks may be more resistant to parasites because they have greater genetic variation at the major histocompatibility complex (MHC; Dunn et al. 2013), which is an important component of the adaptive immune system (Klein 1986). Despite this, a previous study found no relationship between haemosporidian parasites and male ornaments at the between-individual level in common yellowthroats (Dunn et al. 2013). Here we test both the between- and within-individual prediction that ornament expression will be negatively related to parasitism. Males molt and regrow plumage ornaments once a year at the end of the breeding season. Therefore, we measured infection intensity and ornamentation of individuals over two breeding seasons which provided the repeated measures necessary to test the within-individual relationship.

Material and methods

General methods

We captured male common yellowthroats during the breeding season (May–Aug) at the Univ. of Wisconsin-Milwaukee Field Station in Saukville, WI (43°23′N, 88°01′W) in 2003–2004 and 2012–2014. When possible, males were captured in two consecutive years (i.e. 2003–2004, 2012–2013, 2013–2014). During the first capture, we marked each individual with a USFWS metal leg band and three colored plastic leg bands in a unique combination. During every capture we measured tarsus length, wing chord (± 0.1 mm) and body mass (± 0.1 g), took video images to measure mask and bib size, plucked four feathers from the breast to measure color, and took a blood sample for parasite analysis (see below).

To measure the size of the mask and bib, we took a video of each side of the head and the bib with males held against a 1 cm^2 grid. We used still frames from these videos to determine the size of the mask and bib in ImageJ (<http://imagej.nih.gov/ij/>). Each image was scaled to the grid and then ornaments were traced to determine the area (Thusius et al. 2001). To measure the color of the yellow bib, we plucked four feathers from the center of the bib, overlapped them on a black matte background, and measured color using a spectrometer (USB2000, Ocean Optics, Dunedin, FL). For each male, we measured UV brightness (average R from 320–400 nm), yellow brightness (average R from 550–625 nm), yellow saturation (sum of R from 550–625 nm/total R), and carotenoid chroma (Ccar; (R 700–450 nm)/R 700 nm; Andersson and Prager 2006). These color variables are affected by carotenoid concentration, specifically lutein (Andersson and Prager 2006), which is the main carotenoid found in the yellow plumage of common yellowthroats (McGraw et al. 2003).

We collected a small (~70 μl) blood sample from the brachial vein in a heparinized capillary tube. A drop was used to make a blood smear to determine haemosporidian intensity and the remainder was centrifuged to measure hematocrit (% red blood cells). Packed cells were stored in Queen's Lysis Buffer (QLB; Seutin et al. 1991) at 4°C and DNA was extracted from these samples to determine presence and identity of haemosporidian parasites (*Plasmodium* spp. and *Haemoproteus* spp.) in the blood.

Detection and identification of haemosporidian parasites

Blood smears

We determined the intensity of haemosporidian infection using blood smears. Smears were prepared in the field using a small drop of blood, allowed to air dry, and then fixed in methanol for 30 s. Similar to previous studies of avian blood parasites (Giammarino et al. 2007, Levin et al. 2013) we stained slides using a Dip Quick Stain kit (Jorgensen Laboratories, Loveland, CO), which is similar to the Wright–Giesma method. We then mounted a coverslip on each slide using Permount adhesive (Thermo Fisher Scientific, Waltham, MA). For each individual, we scanned approximately 4000 red blood cells under 1000 × oil immersion magnification to estimate the intensity of intracellular haemosporidian parasites. We quantified intensity of infection as the number of cells infected per 4000 (Godfrey et al. 1987).

Nested PCR

To determine the presence of parasites, we extracted DNA from whole blood using a GeneJET Whole Blood Genomic DNA Purification Mini Kit (Thermo Fisher Scientific, Waltham, MA). The genomic DNA extracted from these samples included DNA from both bird and parasites. We determined infection status (i.e. presence or absence of

haemosporidian parasites) using a nested PCR (Hellgren et al. 2004) which amplifies a 520 bp fragment of a conserved region of the cytochrome *b* gene of *Plasmodium* and *Haemoproteus* spp. The first PCR included 1 µl of DNA (~25 ng of genomic DNA), 1.6 µM of forward primer (HaemNF1), 1.6 µM of reverse primer (HaemNR3), 1.5 mM of $MgCl_2$, 5 µl of 5X GoTaq Flexi buffer, 200 µM dNTPs, and 1 U GoTaq DNA polymerase in a total reaction volume of 25 µl. The second PCR used 1 ul of product from the first PCR and the HaemF/HaemR2 (Bensch et al. 2000) primers with the same reagents and concentrations as the first PCR. We used the same thermal profiles as Hellgren et al. (2004) for both PCRs. For each PCR, we included a positive control from an individual known to be infected with haemosporidian parasites and a negative control. To determine if parasite DNA amplified in the sample we ran each PCR product on a 2% agarose gel. We considered samples positive for infection if they had a fragment around 520 bp. For negative samples (i.e. had no fragment at 520 bp) the nested PCR reaction was repeated two more times with the amount of DNA doubled each time. Only samples with three negative PCRs were considered negative for haemosporidian blood parasites.

Identification of parasite lineages

We identified the lineage of parasites by sequencing the PCR product (the parasite cytochrome *b* gene) of positive individuals in both directions. Sequencing was performed at the University of Chicago Cancer Research Center DNA Sequencing Facility. We used Geneious 7.1.9 software (Biomatters, <www.geneious.com/>, Kearse et al. 2012) to align forward and reverse sequences and the BLAST tool on the NCBI website to compare our cytochrome b sequences with those previously characterized by Pagenkopp et al. (2008). All of our parasites were *Plasmodium* (see Results) and the numbering of their lineages follows Pagenkopp et al. (2008).

Survival and breeding experience

We banded all males in the study area in 2003 and 2012 and all new males in 2004 and 2013–2014. We determined putative overwinter survival and breeding experience by surveying the territory and the surrounding area (a minimum of three times May–June) where males were captured the previous year. Males typically return to the same or a nearby territory as the one they occupied the previous breeding season (Taff et al. 2013). Thus, newly banded males in 2004 and 2013–2014 were considered inexperienced breeders and males that did not return to the study area were assumed to not have survived.

Feather growth rate

Parasitism may also affect the condition of birds and, hence, the rate at which they grow new feathers (Coon et al. 2016). To measure feather growth rate, we plucked the third tail feather (R3), counting inward from the outermost right tail feather, and estimated feather growth rate by measuring the average growth bar width on each tail feather. Growth bars are alternating dark and light markings on the feather vane, perpendicular to the shaft (see Fig. 1 in Gienapp and Merilä 2010), and are typically found on wing and tail primaries. A single set of dark and light bars represents 24 h of feather growth. Thus, wider growth bars indicate more feather growth during a 24 h period (Michener and Michener 1938). In addition, growth bar width is positively correlated with nutritional state (Grubb 1989, 1991) and ornament expression (Murphy and Pham 2012). To measure growth bars, we mounted each tail feather on a block of foam covered in black paper and pushed a thin metal pin through the paper at the beginning and end of five growth bars (Hill and Montgomerie 1994, Frasz et al. 2014). We measured the width of the first five growth bars that were visible from the distal end of the feather (Saino et al. 2013) using digital calipers ($\pm$ 0.01 mm). Feather growth rate was expressed as the average width of these five growth bars.

Data analysis

Using nested PCR, we measured presence of parasites for 172 males. For 128 of these 172 males, we determined infection intensity with microscopic examination of blood smears. We had repeated data on infection intensity (in two consecutive breeding seasons) for 28 of the 128 males. Of these 28, we measured mask size for all males, bib size for 27 males, and bib color for 17 males. Finally we measured feather growth rate in 30 individuals, hematocrit in 107 individuals, and survival in 82 individuals. As a consequence, sample sizes differ across statistical models (Table 1–5). Degrees of freedom for each model are based on the number of individuals, rather than the number of observations. We included all available individuals (including uninfected males) in our models. All statistical analyses were performed in JMP 12 (SAS Inst.).

We used within-subject centering to examine the relationships between plumage ornamentation and infection intensity both between- and within-individuals (van de Pol and Wright 2009). To examine whether infection intensity predicts plumage ornamentation across individuals we compared the mean infection intensity for each individual (between-individual slope, β_B). To examine how changes in plumage ornamentation within-individuals were related to variation in infection intensity we examined the mean-centered infection intensity for each individual (within-individual slope, β_W). Mean-centered infection intensities were calculated by finding the average infection intensity for each individual and subtracting this number from each measure of infection intensity for that individual. Our mixed models included measurements of plumage ornament size or color as the dependent variable and the mean infection intensity and mean-centered infection intensity as predictors. To determine if the between-individual slope (β_B) was significantly different from the within-individual slope (β_W), we used mixed models that included mean infection intensity and infection intensity as predictors. In these models, the mean infection intensity term tests for a difference between the within- and between-individual slopes ($\beta_B - \beta_W$) and the infection intensity term tests for a significant negative or positive slope within-individuals (β_W). Individual identity was included in both models as a random factor. We ran separate models for bib size and the components of bib color

Table 1. Between-individual (β_B) and within-individual (β_W) relationships between plumage ornaments and intensity of haemosporidian infection. The within-individual relationship was based on changes between breeding seasons in the same individual.

		Estimate	SE	*t*	df	p
Mask size	Intercept	329.60	7.66	43.04		<0.01
	Mean infection intensity (β_B)	–0.03	0.29	0.11	1,25	0.92
	Centered mean infection intensity (β_W)	0.85	0.39	2.15	1,25	**0.04**
Bib size	Intercept	1055.38	52.68	20.04		<0.01
	Mean infection intensity (β_B)	1.39	1.96	0.71	1,24	0.49
	Centered mean infection intensity (β_W)	–1.37	3.28	0.42	1,24	0.68
UV brightness	Intercept	21.40	1.18	18.12		<0.01
	Mean infection intensity (β_B)	–0.07	0.04	1.90	1,14	0.08
	Centered mean infection intensity (β_W)	–0.11	0.05	2.31	1,14	**0.03**
Yellow brightness	Intercept	30.20	1.06	28.45		<0.01
	Mean infection intensity (β_B)	–0.05	0.03	1.44	1,14	0.17
	Centered mean infection intensity (β_W)	–0.09	0.05	1.64	1,14	0.12
Saturation	Intercept	0.27	0.01	49.11		<0.01
	Mean infection intensity (β_B)	<0.01	<0.01	1.40	1,14	0.18
	Centered mean infection intensity (β_W)	<0.01	<0.01	2.02	1,14	0.06
Carotenoid chroma	Intercept	0.78	0.02	35.68		<0.01
	Mean infection intensity (β_B)	<0.01	<0.01	0.76	1,14	0.46
	Centered mean infection intensity (β_W)	<0.01	<0.01	1.70	1,14	0.11

because these traits are independently related to different aspects of male quality (Freeman-Gallant et al. 2010, 2011, Taff et al. 2012, Taff and Freeman-Gallant 2014, Henschen et al. 2016), although some components of these ornaments are correlated with each other.

To determine if feather growth rate or hematocrit were related to infection presence or intensity we used general linear models (GLM) that included hematocrit or feather growth rate as the dependent variable and year, date of capture, tarsus length, body mass, breeding experience and either presence of infection or infection intensity as predictors. We also used within-subject centering to determine if there was a within-individual relationship between infection intensity and hematocrit. Lastly, we performed nominal logistic regressions to determine if survival (yes/no) was related to the presence or intensity of parasite infection. These models included survival as the dependent variable and year, date of capture, tarsus length, body mass, breeding experience and either presence of infection or infection intensity as predictors.

Data deposition

Data available from the Dryad Digital Repository: <http://dx.doi.org/10.5061/dryad.5cj78> (Henschen et al. 2017).

Results

Presence, intensity, and lineage of haemosporidian parasites

We determined the presence of haemosporidian parasites for 172 individuals using a nested PCR and found that 83% of individuals were positive for these parasites. Based on our analysis of blood smears, 82 of 128 (64%) individuals were infected with haemosporidian parasites. Both methods (n = 120) gave the same result for 72% of individuals (11 negative, 75 positive), but differed for the other 34 individuals (4 were positive only with the blood smear method and 30 were positive only with the PCR method). Infection intensity (number of cells infected per 4000) ranged from 0 to 3.1% of cells (mean = 0.26%). We sequenced the cytochrome *b* gene of haemosporidian parasites for 117 males with a positive PCR product. All parasites shared 100% sequence identity with five previously characterized lineages of the *Plasmodium* genus, except for three samples that varied at one or two bases. Most (70%; 82/117) individuals were infected with lineage 6a (Genbank: EU328172) as described by Pagenkopp et al. (2008). The next most common lineages were 4b (21/117; EU328173) and 6b (11/117; EU328171). Lineages 1 (EU328168) and 5

Table 2. Difference between the slopes for between- and within-individual relationships ($\beta_B - \beta_W$) of haemosporidia parasite infection intensity (number of cells infected) with mask size, bib UV brightness, and bib saturation.

	Term	Estimate	SE	*t*	df	p
Mask size	Intercept	329.60	7.66	43.04		<0.01
	Mean infection intensity ($\beta_B - \beta_W$)	–0.88	0.49	1.80	1,25	0.08
	Infection intensity (β_W)	0.85	0.39	2.15	1,25	**0.04**
UV brightness	Intercept	21.40	1.18	18.12		<0.01
	Mean infection intensity ($\beta_B - \beta_W$)	0.04	0.06	0.71	1,14	0.48
	Infection intensity (β_W)	–0.11	0.05	2.31	1,14	**0.03**
Saturation	Intercept	0.27	0.01	49.11		<0.01
	Mean infection intensity ($\beta_B - \beta_W$)	< –0.01	<0.01	0.39	1,14	0.70
	Infection intensity (β_W)	<0.01	<0.01	2.02	1,14	0.06

Table 3. Relationship between hematocrit and the presence or absence of haemosporidia parasites (based on PCR), or haemosporidia infection intensity (number of cells infected). Year was included as a categorical variable in this model.

	Term	Estimate	SE	*t*	p
Hematocrit	Intercept	52.66	19.20	2.74	0.01
	Year[2004–2003]	0.12	1.46	0.08	0.94
	Year[2005–2004]	2.50	2.49	1.00	0.32
	Year[2006–2005]	–2.84	2.81	1.01	0.31
	Year[2013–2006]	0.62	1.99	0.31	0.76
	Year[2014–2013]	0.17	1.73	0.10	0.92
	Capture date	–0.12	0.03	3.69	**<0.01**
	Breeding experience	0.22	0.59	0.38	0.71
	Tarsus length	–0.21	0.92	0.22	0.82
	Body mass	0.58	1.11	0.52	0.60
	Infection presence	1.24	0.75	1.65	0.10
	$R^2 = 0.19$, $F_{10,97} = 2.21$, $p = 0.02$				
Hematocrit	Intercept	52.89	20.79	2.54	0.01
	Year[2004–2003]	–0.86	1.52	0.57	0.57
	Year[2013–2004]	1.50	1.81	0.83	0.41
	Year[2014–2013]	–0.48	1.98	0.24	0.81
	Capture date	–0.12	0.03	3.71	**<0.01**
	Breeding experience	0.62	0.67	0.92	0.36
	Tarsus length	0.09	1.01	0.09	0.93
	Body mass	–0.14	1.23	0.12	0.91
	Infection intensity	<0.01	0.03	0.17	0.87
	$R^2 = 0.25$, $F_{8,69} = 2.85$, $p = 0.01$				

(EU328175) were the least common, detected in one and two individuals, respectively. There was no evidence of multiple infections. The corresponding sequences in the Malavi database (<http://mbio-serv2.mbioekol.lu.se/Malavi/index.html>) are: 1 = TABI08, 4b = SEINOV01, 5 = BT7, 6a = GEOTRI01 and 6b = BAEBIC02.

Haemosporidian infection and ornamentation

Between-individuals

At the between-individual level males with greater bib UV brightness tended to have fewer infected red blood cells than males with lower bib UV brightness (β_B; Table 1). No other measures of ornamentation were related to infection intensity at the between-individual level (β_B; Table 1).

Within-individuals

When individuals were measured over two breeding seasons, infection intensity was related to several measures of plumage ornamentation within-individuals. Males that had an increase in infection intensity from one breeding season to the next also had an increase in mask size (β_W, Fig. 2, Table 1), and they tended to have an increase in bib saturation (β_W, Table 1). In contrast, an increase in infection intensity between years was related to a decrease in UV brightness (Table 1). No other measures of plumage ornamentation (i.e. bib size, yellow brightness, or carotenoid chroma) were related to changes in infection intensity within-individuals (Table 1). For mask size, the between-individual slope (β_B) tended to differ ($p = 0.08$) from the within-individual slope, but there was no difference for any of the other plumage traits ($\beta_B - \beta_W$; Table 2).

Feather growth rate, hematocrit and survival

Infection intensity was not related to hematocrit either between- ($p > 0.10$; Table 3) or within-individuals ($p > 0.58$; Table 4). In between-individual analyses, neither the presence nor the intensity of haemosporidian infection was related to feather growth rate (average growth bar width; $p > 0.66$; Table 5) or survival (all $p > 0.85$; Table 6).

Discussion

In common yellowthroats, we found differences in the relationship between haemosporidian infection and plumage ornamentation at the within- and between-individual levels. At the between-individual level (i.e. when we compared individuals across the population) the size and yellow coloration of plumage ornaments (black mask and yellow bib) was not related to the intensity of blood parasite infection. Thus, our results do not support the between-individual prediction of the Hamilton–Zuk hypothesis; i.e. males with the lowest parasitism will have the most elaborate ornaments. However, plumage ornaments (i.e. mask size and bib UV

Table 4. Between-individual (β_B) and within-individual (β_W) relationships between haemosporidian infection and hematocrit. The within-individual relationship was based on changes in hematocrit and infection intensity in individuals over two separate breeding seasons.

		Estimate	SE	*t*	df	p
Hematocrit	Intercept	45.64	1.59	28.63		<0.01
	Mean infection intensity (β_B)	0.15	0.11	1.37	1,11	0.20
	Centered mean infection intensity (β_W)	0.04	0.08	0.56	1,11	0.58

Table 5. Relationship between tail feather growth rate (average growth bar width) and the presence or absence of haemosporidia parasites (based on PCR), or haemosporidia parasite infection intensity (number of cells infected).

	Term	Estimate	SE	*t*	p
Feather growth rate	Intercept	–1.35	2.39	0.57	0.58
	Year	–0.09	0.11	0.79	0.44
	Capture date	< –0.01	0.01	0.08	0.94
	Tarsus length	0.04	0.10	0.39	0.70
	Body mass	0.31	0.18	1.68	0.11
	Breeding experience	–0.05	0.06	0.75	0.46
	Infection presence	–0.03	0.07	0.44	0.66
	$R^2 = 0.16$, $F_{6,24} = 0.76$, $p = 0.61$				
Feather growth rate	Intercept	–0.66	2.66	0.25	0.81
	Year	0.11	0.13	0.85	0.41
	Capture date	< –0.01	0.01	0.63	0.54
	Tarsus length	< –0.01	0.11	0.01	0.99
	Body mass	0.33	0.19	1.73	0.10
	Breeding experience	–0.02	0.07	0.31	0.76
	Infection intensity	< –0.01	<0.01	0.33	0.75
	$R^2 = 0.19$, $F_{6,16} = 0.63$, $p = 0.70$				

brightness) were related to infection intensity at the within-individual level. Here, we discuss potential reasons why there are within-individual, but not between-individual, relationships between ornaments and parasitism.

The lack of a between-individual relationship between parasites and ornaments was surprising, because there is some evidence that male common yellowthroats with larger black masks have better immunity (e.g. greater diversity of MHC class II alleles) than males with smaller masks (Dunn et al. 2013). However, there are a number of factors that can obscure the predicted relationship at the between-individual level and lead to discrepancies between results of the two types of analyses. In particular, between-individual studies may include males of different ages, and age can independently affect both parasitism and the expression of ornaments.

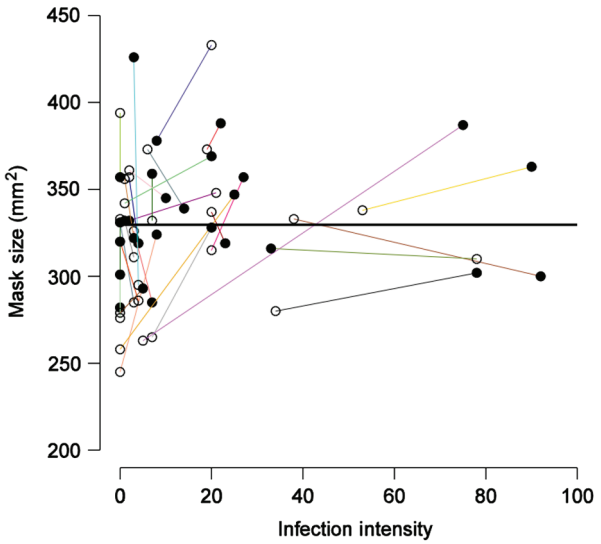

Figure 2. Relationship between mask size (mm^2) and intensity of infection by haemosporidia (number of cells infected). Thin lines represent within-individual slopes and each color represents a different individual. The thick line represents the between-individual slope. Open circles are the first year of capture and closed circles are the second year of capture.

For example, older individuals are potentially more likely to be infected by virtue of their longer exposure (longer lifetime), and they are more likely to be resistant by virtue of their survival to an older age (Fig. 1). Older males may also express a more elaborate ornament because it is favored by sexual selection (Kokko 1997), or because they have more experience, which improves their ability to acquire resources for ornament production (Daunt et al. 2007, Zimmer et al. 2011). Between-individual relationships may also be masked because individual parasitism can change between seasons and years (Hegemann et al. 2012), but static ornaments, such as plumage, only reflect levels of parasitism at the time they were produced and not necessarily when the parasites were sampled. This may be a widespread problem in studies of plumage ornaments as parasitism is often measured during the breeding season, but ornaments are often produced during the previous spring or autumn molt. For example, in house finches, plumage redness was not related to mite load before molt (i.e. between-individuals), but males with fewer mites had a greater increase in plumage redness after molt (i.e. within-individuals; Thompson et al. 1997). In this study the between-individual relationship may be obscured because we measured both ornamentation and infection intensity during the summer breeding season (May–Aug), although the plumage ornaments of common yellowthroats are produced during the autumn molt.

Although the Hamilton–Zuk hypothesis predicts a negative within-individual relationship between ornamentation and parasitism, male common yellowthroats actually showed the opposite relationship, an increase in both mask size and parasitism between years. An age-related increase in parasite tolerance could produce this positive within-individual relationship. For example, males could gain access to more resources as they age if they obtain better territories or learn to forage more efficiently (Daunt et al. 2007, Zimmer et al. 2011). Thus, older males might have more energy or nutrients available to both increase ornamentation and tolerate an increase in parasitism (Morehouse 2014). Another possibility is that haemosporidian infection is not very costly. In our study, the infection intensity (i.e. percent of red blood cells infected) of all but one individual was less than 2%, which

Table 6. Relationship between overwinter survival (in 2004, 2013–2015) and the presence or absence of haemosporidia parasites (based on PCR), or haemosporidia infection intensity (number of cells infected).

		Estimate	SE	ChiSquare	p
Survival	Intercept	8.99	10.17	0.78	0.38
	Year[2004–2003]	1.64	0.70	5.50	**0.02**
	Year[2005–2004]	–1.65	0.92	3.20	0.07
	Year[2013–2005]	0.02	1.03	< 0.01	0.99
	Year[2014–2013]	0.47	0.90	0.28	0.60
	Capture date	–0.02	0.02	1.31	0.25
	Tarsus length	0.09	0.49	0.04	0.85
	Body mass	–0.98	0.63	2.39	0.12
	Breeding experience	–0.12	0.34	0.14	0.71
	Infection presence	0.07	0.36	0.03	0.85
	$R^2 = 0.11$, $ChiSqr_{9,73} = 12.63$, $p = 0.18$				
Survival	Intercept	10.41	12.67	0.68	0.41
	Year[2004–2003]	1.87	0.87	4.62	**0.03**
	Year[2013–2004]	–3.71	1.35	7.5	**0.01**
	Year[2014–2013]	3.70	1.77	4.37	**0.04**
	Capture date	–0.01	0.02	0.13	0.72
	Tarsus length	0.74	0.64	1.3	0.25
	Body mass	–2.41	0.91	7.09	**0.01**
	Breeding experience	–0.21	0.40	0.29	0.59
	Infection intensity	< 0.01	0.03	0.03	0.86
	$R^2 = 0.28$, $ChiSqr_{8,52} = 23.43$, $p = < 0.01$				

suggests that these are chronic, rather than acute, infections (Valkiūnas 2004). In addition, parasites did not affect survival, hematocrit, or feather growth rate, further suggesting that these parasites were not costly. In this case, there may be no direct relationship between ornamentation and parasitism; they each increase with age for different reasons. For example, ornament expression may increase if ornaments are sexually selected indicators of age (Kokko 1997, Brooks and Kemp 2001, Marini et al. 2015), and parasitism may increase with age simply due to increased exposure to infection. In common yellowthroats mask size is a sexually selected trait through both within- and extra-pair mate choice, and it also increases with age (Thusius et al. 2001). Thus, the benefits of increased mating success for older males with larger masks likely outweighs the apparently minimal costs imposed by a low-level infection. Finally, it is also possible that older males invest more resources in reproduction (i.e. ornament expression) at the expense of self-maintenance (i.e. immune response; Williams 1966, Velando et al. 2006).

Similar to melanin-based ornaments, an increase in parasitism is predicted to negatively affect the expression of carotenoid-based ornaments. In particular, parasitism is thought to decrease the availability of carotenoids for ornamentation, as more carotenoids will be needed for regulating oxidative stress induced by parasites (von Schantz et al. 1999, Mougeot et al. 2010). In addition, carotenoids can influence the immune response itself (Lozano 1994, Blount et al. 2003, Aguilera and Amat 2007), as they can stimulate both the humoral and the cell-mediated immune response (reviewed by Chew and Park 2004). In our study, we found that UV brightness was negatively related to parasitism at the within-individual level, while bib saturation tended to be positively related to parasitism. These seem like opposing relationships, but it is important to note that carotenoid (lutein) concentration in feathers affects UV brightness and saturation in opposite ways – as carotenoid concentration increases, saturation increases, while UV brightness decreases (Andersson and Prager 2006). Thus, this suggests that the concentration of carotenoids in the yellow bib feathers is actually related positively to parasitism. This is consistent with the positive within-individual relationship between mask size and parasitism. In yellowthroats, these relationships may be due to age-related factors, such as increased foraging ability, and, thus, experiments may be necessary to determine any causal relationships between pigments (carotenoids and melanins) and parasitism.

Overall, we found that more elaborate plumage ornaments did not signal lower haemosporidian infection in male common yellowthroats at the between-individual level, and, at the within-individual level, mask size was positively related to greater infection intensity. Carotenoid concentration in the breast feathers, as indexed by UV brightness and saturation, was also related to greater infection intensity. These relationships were only evident in within-individual analyses, which highlights the importance of this type of analysis for understanding physiological tradeoffs that underlie population-wide patterns.

Acknowledgements – We thank two anonymous reviewers for helpful comments on the manuscript. We also thank the staff of the Univ. of Wisconsin-Milwaukee (UWM) Field Station, C. Campeau, A. Dellemann, J. Garvin, J. Kilmer, J. Maurer, H. Owen, M. Petersen, R. Schneider, and M. Snow for assistance in the field or lab.
Funding – Funding was provided by a UWM Research Growth Initiative grant to LAW and POD, and grants from the UWM Department of Biological Sciences, the Animal Behavior Society, the American Ornithologists' Union, and the Wisconsin Society for Ornithology to AEH.
Conflicts of interest – The authors declare no conflict of interests.
Permit – This research was approved by the UWM Animal Care and Use Committee (Permit 11-12 #33).

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