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GUT PARASITE LEVELS ARE ASSOCIATED WITH SEVERITY OF RESPONSE TO IMMUNE CHALLENGE IN A WILD SONGBIRD

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ABSTRACT: Life history trade-offs have been posited to shape wild animals' immune responses against microparasites (e.g., bacteria, viruses). However, coinfection with gut helminths may bias immune phenotypes away from inflammatory responses and could be another mechanism underlying variation in immune responses. We examined how the magnitude of a common and costly response to microparasites, the acute phase response (APR), varied with helminth coinfection at both the individual and the population levels in Song Sparrows (*Melospiza melodia*). The APR includes fever and sickness behaviors, like lethargy and anorexia, and provides a whole-organism metric of immune activation. We combined data on fever and lethargy in response to an immune challenge (lipopolysaccharide) with postmortem data assessing helminth burdens and data on malarial parasite infection from blood samples in sparrows from two populations: southern California and western Washington, USA. We predicted that birds with higher helminth burdens would express less severe APRs, at both the individual and population levels. Furthermore, we predicted that these reduced immune responses would diminish resistance against malarial parasites and would thus be associated with higher prevalences of such parasites. Previously, Song Sparrows from Washington have been shown to mount less severe APRs than those from California. In our study, Washington birds also exhibited higher helminth burdens and a higher prevalence of one type of avian malarial parasite. Because of low variation in helminth burdens in California (median=0, range=0–3), we tested within-population relationships only in birds from Washington, where the severity of fever and lethargy correlated negatively with helminth burden. These results suggested that helminth coinfection could help mediate immune responsiveness in wild songbirds.

Key words: Acute-phase response, fever, helminth, Song Sparrow, sickness behavior.

INTRODUCTION

Immune responses vary greatly among avian individuals, populations, and species, with implications for how infectious diseases spread and evolve in the wild (Owen-Ashley and Wingfield 2007; Brock et al. 2014; Downs et al. 2014). One possible reason for immune variability is that mounting an immune response incurs significant costs (e.g., energy, amino acids, time, and damage to a host's own tissue) and thus represents a trade-off between resources used for reproduction and resources used for maintenance (Sheldon and Verhulst 1996). A second, less explored possibility suggests that prior infection with parasitic worms (helminths) may drive variation in immune responses to subsequent infections (Maizels et al. 2004). Understanding how helminths affect an organism's immune response may help predict the risks posed to that organism from other parasites, including microparasites.

The acute phase response (APR) is an immune response triggered by diverse microparasites and involves activation of Toll-like receptors, induction of proinflammatory cytokine signaling, the production of antimicrobial peptides, and whole-body responses, including fever, lethargy, and anorexia (Owen-Ashley and Wingfield 2007). Helminths can alter these host immune responses using diverse mechanisms, including induction of T regulatory cells, which can dampen proinflammatory signaling, and biasing helper T cells (Th) toward type 2 (Th2) phenotypes

(Maizels et al. 2004). These Th2 phenotypes typically include the production of anti-inflammatory cytokines, including interleukin (IL)-3, -4, -21, and -25, and function to facilitate immune responses against extracellular parasites, notably favoring production of specific antibody subtypes (Anthony et al. 2007). Additionally, cytokines secreted by Th2 cells limit differentiation of Th cells into Th1 phenotypes, which typically respond to microparasites such as bacteria and viruses (Anthony et al. 2007). The Th1 responses are characterized by proinflammatory cytokines (e.g., interferons, IL-2, tumor necrosis factor [TNF]-α) and facilitate whole-organism responses to infection, including fever and sickness behaviors like lethargy and anorexia (Ashley et al. 2012). At least two of these Th1 cytokines, TNF-α and IL-1β, help mediate production of IL-6, a cytokine associated with changes to thermoregulation in response to injection with lipopolysaccharide (LPS), a cell wall component of gram negative bacteria (Shalaby et al. 1989). However, immune modulation by helminths can be highly variable (Anthony et al. 2007). For example, *Ascaridia galli*, a commercially destructive nematode, increases intestinal inflammation in laying hens (*Gallus domesticus*), and *Litomosoides sigmodontis*, a filarial nematode of mice (*Mus* spp.), reduces systemic inflammation during sepsis (Marcos-Atxutegi et al. 2009; Gondorf et al. 2015). Such complexity of helminth–immune response interplay highlights the need for further investigations in wild vertebrates, particularly wild birds, where little is known about helminth-induced immunomodulation (Pedersen and Babayan 2011).

We asked whether helminth infection helped to shape immune responses to microparasites in a widespread North American songbird, the Song Sparrow (*Melospiza melodia*). Previous field and common garden experiments show that fever, sickness behaviors, and IL-6 production, key components of the APR, are more pronounced in Song Sparrows from southern California than from Washington (Adelman et al. 2010a, b). This pattern could be consistent with different relative costs of the APR between populations, specifically that reducing the energetic and time costs of the APR is crucial in Washington because of a shorter, more intense breeding season (Adelman et al. 2010b). However, immunomodulation by helminths remains an unexplored potential driver in this system.

We combined data on helminth loads with previously published data from a common garden experiment on the APR in sparrows from southern California and Washington (Adelman et al. 2010a). Because the data on immune responsiveness were collected in a common laboratory environment, confounding effects of different habitats (e.g., arid southern California vs. mesic western Washington), with potential differences in resource availability, are minimized (Adelman et al. 2010a). Additionally, we incorporated data on a common set of blood microparasites, the avian malaria parasites *Plasmodium* sp. and *Parahaemoproteus* spp., to determine whether increasing helminth burdens correlated with the risk of microparasite infection in the wild. Because helminths induce Th2-type immune responses (Anthony et al. 2007), often reducing APRs in mice and humans (Maizels et al. 2004), we predicted that the APR should vary inversely with helminth burden, at both the individual and the population levels. Additionally, because vertebrate defenses against malaria parasite infection often include the APR (Williams 2005; Bichet et al. 2012), we predicted that, if APRs are reduced under higher helminth burdens, then malaria parasite prevalence should be higher.

MATERIALS AND METHODS

Source data

Data on immune responses used in the present analyses were originally collected in March–May 2009 and were previously published (Adelman et al. 2010a). Data on gut helminth burdens, prevalence of malaria parasites, and their relationships with immune responses were collected afterward and are published here for the first time. Adelman et al. (2010a) provide detailed information on capture, housing, and immune treatments. We briefly outline those aspects of the

study here, concentrating in more detail on parasite assessments and statistical analyses.

Study species, field capture, and permitting

Adult male Song Sparrows were brought into a common captive environment from two populations on the western coast of North America: one in southern California, USA, the other in western Washington, USA. In southern California, birds of the *M. melodia fallax* subspecies were captured using mist nets and song playback at the Sonny Bono Salton Sea National Wildlife Refuge and Imperial Wildlife Management Area (33°16′18″N, 115°34′49″W). In western Washington, birds of the *M. melodia morphna* subspecies were captured using the same methods at the Charles L. Pack Experimental Forest (46°50′41″N, 122°17′32″W). These two sites experience very different climates. Data from the nearest National Oceanic and Atmospheric Administration weather stations (Imperial, California, about 40 km from our site; Mowich, Washington, about 30 km from our site) showed that average daily high temperature during March–May from 1999 to 2008 was 30.5 C (SD=5.6 C) near our California site and 10.7 C (SD=5.6 C) near our Washington site (National Oceanic and Atmospheric Administration 2018). During this same period, total precipitation from March to May averaged 8.4 mm (SD=7.0 mm) near our California site and 638.2 mm (SD=50.4 mm) near our Washington site (National Oceanic and Atmospheric Administration 2018). Birds at both locations were most often detected in low, scrubby vegetation, typically *Tamarisk* spp. in California and *Rubus* spp. in Washington, frequently near permanent water sources. Captures occurred during the early breeding season at each location (California: 6–12 March 2009; Washington: 11–16 April 2009). After being temporarily housed in nylon tents at each field site, birds were transported to Princeton University via commercial aircraft. All work was conducted under the following federal, state, and institutional permits: US Geological Survey Bird Banding Laboratory permit 22965, US Fish and Wildlife Service scientific collecting permit MB026193-0, California scientific collecting permit SC-009218, and Princeton University Institutional Animal Care and Use Committee protocol 1745.

Housing and immune challenge

In captivity, birds were housed in individual cages (25×55×25 cm) with two perches. Birds were provided ad libitum access to water, grit, and food (1:1 mix of Kaytee Supreme Finch Food and Mazuri Small Bird Maintenance pellets, Purina Mills, Gray Summit, Missouri, USA). Lights were controlled by a timer set weekly to mimic natural day length at the birds' locations of capture. Rooms were maintained at a constant 23 C.

Before experimentation began, we measured birds' body condition using tarsus length (a measure of structural size) and body mass. Specifically, we performed separate linear regressions within each population with mass as the dependent variable and tarsus length as the independent variable, taking the residuals from this analysis as our measure of body condition. As such, a positive residual indicated that a bird had relatively more physiologic reserves (mass) than would be predicted for its structural size (tarsus length).

Experiments were begun after 2 wk of acclimation, treating two to four birds each day. Birds were captured, weighed, and fitted with a temperature-sensing radio transmitter (LB-2NT, Holohil Systems Ltd., Carp, Ontario, Canada), as described in Adelman et al. (2010a). A coin toss determined whether the first bird would be either handled and left uninjected (control group) or injected subcutaneously over the breast muscle with lipopolysaccharide mixed 1:1 with Freund's incomplete adjuvant (LPS group, LPS: cat. L2880, serotype 055:B5, Sigma-Aldrich, St. Louis, Missouri, USA; Adjuvant: cat. F5506, Sigma-Aldrich). The final concentration of LPS stock was 2 mg/mL, and injections were adjusted for the birds' weights to yield a final dose of 2.1 μg LPS/g body mass (Adelman et al. 2010a, b).

We monitored birds for fever (change in skin temperature) and reductions in activity level (which correspond directly with lethargy), components of the APR, using automated radio telemetry receivers (model 10-1000, Sparrow Systems, Champaign-Urbana, Illinois, USA). Receivers recorded one data point per bird each 30 s. Transmitters encoded temperature data by varying the interval between pulses and were calibrated by the manufacturer, with a subset retested in our laboratory to confirm accuracy. We calculated periods of inactivity as any time signal strength from the transmitter remained within ±4 dB for 1 min or more (Adelman et al. 2010a, b).

We also took blood samples from animals at either 6 or 22 h posttreatment for analysis of IL-6–like bioactivity. Blood was drawn from the wing vein via venipuncture and collected in heparinized capillary tubes before centrifugation to separate plasma. Plasma was stored at −20 C until it was tested using a cell culture–based technique to assess IL-6–like bioactivity (Adelman et al. 2010a).

Assessment of gut helminths

At either 6 or 22 h posttreatment, animals were euthanized with an overdose of isoflurane. Livers

and spleens were removed within 15 min of death for a separate experiment. Carcasses were then frozen at –20 C for up to 2 yr before being thawed to examine the intestinal tract for the presence of helminths. After being thawed at 4 C, the intestinal tract was slowly opened by cutting from the proximal (stomach) to distal (cloacal) end with blunt-tipped dissecting shears under the dissecting scope, being careful not to cut any visible worms. Once fully opened, we examined the interior of the intestine for helminths using a 10×–40× magnification dissecting scope. Detected helminths were removed and placed in ethanol. Finally, we removed the intestine and poured the water from the petri dish onto a fine mesh (0.42 mm) filter, 7 cm in diameter. This step removed fine debris and allowed a final observation in water of reduced turbidity (better visibility). The intestine was then replaced into the original petri dish, and debris present on the filter was rinsed back into the same dish.

Assessment of malaria parasite infection

To screen for malaria parasites, we first extracted DNA from blood samples using the QIAGEN BioSprint 96 System (Qiagen USA, Germantown, Maryland, USA) following the manufacturer's guidelines. Extracted samples were subjected to a nested PCR using the conserved primers DW2/DW4 and DW1/DW3, which amplify a 614–base pair region of the mitochondrial cytochrome *b* gene (*cytb*) of malaria parasites (Martinsen et al. 2006). Negative and positive controls were included during the PCR, and no contamination was detected. PCR products were visualized by gel electrophoresis. Positive PCR products were purified using ExoSAP-IT (Affymetrix, Thermo Fisher Scientific, Cleveland, Ohio, USA) and sequenced on an ABI 3130*xl* Sequencer (Applied Biosystems, Foster, California, USA) at the Smithsonian Conservation Biology Institute's Center for Conservation Genomics. Sequences were visualized and edited using Sequencher version 5.0 (Gene Codes, Ann Arbor, Michigan, USA) and BLASTed within a malaria parasite dataset (Martinsen et al. 2008) to identify each *cytb* sequence to genus. A BLAST analysis of each unique sequence obtained in this study against all avian malaria parasite sequences within the MalAvi database (Bensch et al. 2009) allowed for identification of malaria parasite lineages.

Statistical analyses and samples sizes

All analyses were performed in R v.3.1.3 (R Development Core Team 2017). Unless otherwise noted, all analyses were performed using generalized linear models (GLM), initially incorporating body condition and its interaction with other independent variables. Interactions were removed by backward elimination if their *P* values were greater than 0.05. Similarly, body condition was removed from final models if its *P* value was greater than 0.05 (as was the case in all but one model). Error distributions used for GLMs varied based on the type of data. When comparing the probability of infection with blood parasites, we assumed quasibinomial errors; when assessing the burden of gut parasites, we assumed negative binomial errors (Venables and Ripley 2002); and when analyzing the effect of infection on immune responses, we assumed Gaussian errors. Confidence intervals for percentages were calculated by the "binom" package in R (Sundar 2014) using the "exact" method.

When comparing the probability of *Parahaemoproteus* infection between populations, our GLM produced highly inaccurate estimates because no California birds were infected with this parasite (standard errors were three orders of magnitude greater than the estimates). Therefore, to compare the probability of being infected with *Parahaemoproteus* between populations, we used a χ^2 test.

We used separate GLMs to compare fever, lethargy, and IL-6–like bioactivity against helminth burdens in LPS-treated birds. Fever was analyzed as the integral over time between a bird's change in skin temperature and the mean change in skin temperature for control birds from the same population of origin, those untreated with LPS (Adelman et al. 2010b). Changes in activity level (to assess lethargy) were analyzed as the proportion of time spent active from hours 1–12, postinoculation.

The experiment from which these data were drawn originally included 29 individuals from California and 27 from Washington. For reasons detailed below, sample sizes were not equivalent for all comparisons (Table 1). Although we sampled all animals to assess infection with blood parasites, we were only able to recover carcasses from 48 animals to assess helminth burdens (26 from California, 22 from Washington). One control bird (not LPS-treated) from Washington was removed from all analyses because of an infection before the start of the experiment. If this bird was included for analyses of blood or gut parasites, results were qualitatively identical.

Because birds from California showed minimal variation in helminth burden among individuals, we relied exclusively on animals from Washington for within-population comparisons involving helminths (Fig. 1; California: median=0 helminths, range=0–3; Washington: median=6 helminths, range=0–80). For these within-population analyses, several additional factors limited our sample sizes. First, we could only use birds treated with

TABLE 1. Sample sizes for between- and within-population comparisons of gut helminth burdens, blood parasite prevalence, fever, lethargy, and IL-6–like bioactivity in Song Sparrows (*Melospiza melodia*). Within-population variability of helminth burden was insufficient to perform within-population comparisons on California birds.

	Between-population comparisons		Within-population comparisons			
	Infection		Helminth burden vs.			
Location	Gut helminth	Blood parasite	Blood parasite infection	Fever	Lethargy	IL-6–like bioactivity
California	26	29	na[a]	na	na	na
Washington	22	26	22 (21[b])	7	14 (13[b])	5
Total	48	55	22 (21[b])	7	14 (13[b])	5

[a] na = not applicable.

[b] n=13 for analyses, excluding outlier.

LPS, because these were the only animals in which we had induced an immune response. Second, the study involved two cohorts, one sacrificed at 6 h posttreatment, the other at 22 h posttreatment. Because fever only began to manifest at roughly 6 h postinoculation (Adelman et al. 2010a), we excluded birds euthanized at 6 h postinoculation when testing the relationship between fever and helminth burden. Because IL-6–like bioactivity is very low and minimally variable at 22 h postinoculation (Adelman et al. 2010a), we used only 6 h postinoculation birds to test the relationships between IL-6 signaling during the APR and helminth burden. To test the relationship between helminth burden and lethargy (1 proportion of time spent active) within the birds from Washington, we included all LPS-treated birds from the Washington population. We performed the analyses of blood parasites vs. helminth burden and lethargy vs. helminth burden with and without an outlier—a single bird whose intestine contained 80 helminths, 13 standard deviations above the mean for all other Washington birds. Finally, because of transmitter malfunctions, which yielded constant temperature readings, but accurate activity readings, temperature data were not available for three Washington birds.

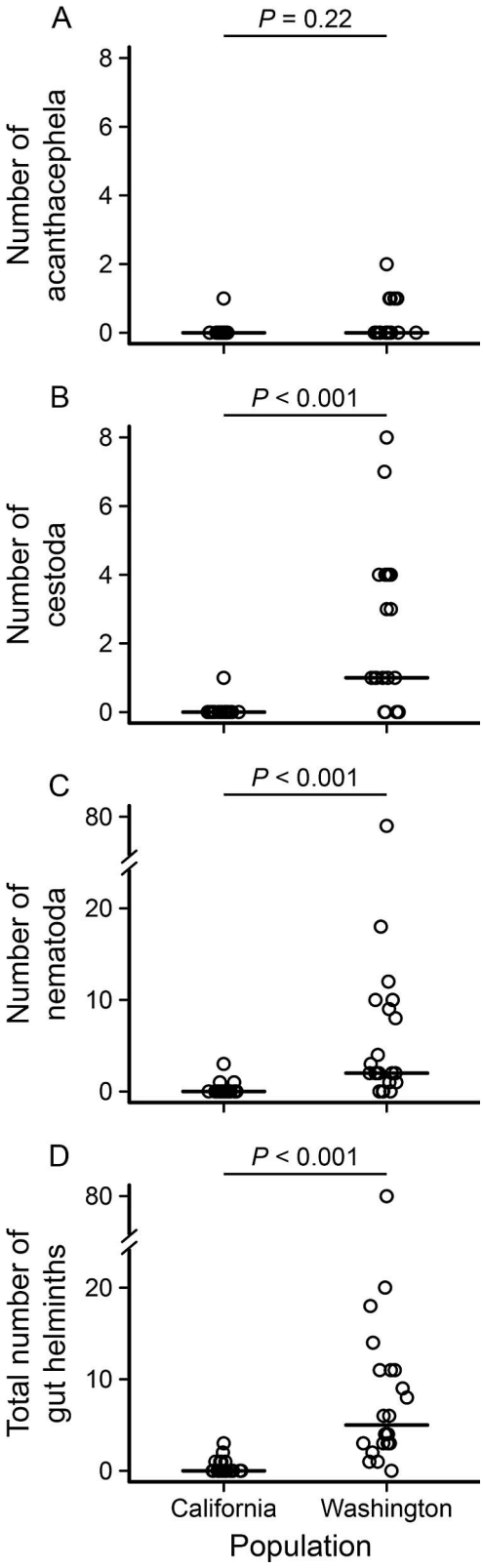
RESULTS

Population differences in parasites

We detected helminth infections in 95% (21/22, 95% CI: 77–99%) of Song Sparrows from Washington. Nematode and cestode infections were equally prevalent, with 77% of birds (17/22, 95% CI: 55–92%) infected with nematodes, cestodes, or both, and Acanthocephala infections were the least common, with only 23% of birds (5/22, 95% CI: 8–45%) infected. In contrast, among Songs Sparrows from California, we detected helminths in only 23% (6/26, 95% CI: 9–44%) of individuals, a significant difference from the Washington sample (χ^2=25.4, df =1, P<0.001). Among the six California birds that harbored helminths, two were infected with acanthocephalans only, two were infected with nematodes only, one was infected with both a nematode and an acanthocephalan, and one harbored a single cestode.

Although Song Sparrows from Washington and California showed similar burdens of Acanthocephala parasites (Fig. 1A; GLM, population_Washington parameter estimate=0.86, SE=0.71, $z_{1,46}$=1.22, P=0.224), birds from Washington had higher burdens of cestodes, nematodes, and total gut helminths than birds from California (Fig. 1B–D; for all GLMs, population_Washington parameter estimate>3.37, SE<1.04, $z_{1,46}$>3.91, P<0.001). Neither body condition nor its interaction with population predicted helminth burdens (all P>0.185) and were thus removed from final models.

Additionally, two genera of malaria parasites were sequenced from the birds: *Plasmodium* and *Parahaemoproteus*. These genera never occurred within the same individual, and only *Plasmodium* parasites were present in California birds, although both genera occurred in Washington (Fig. 2A). All sequenced infections identified to avian malaria parasites were previously documented and



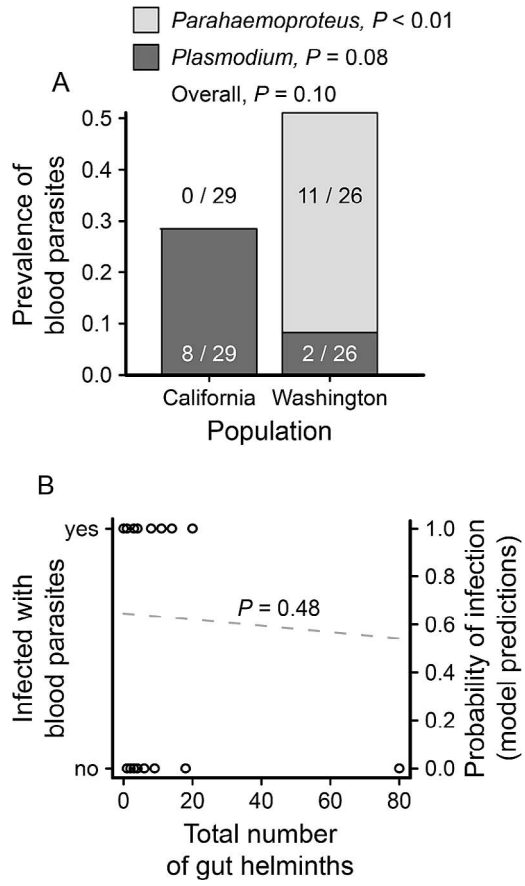


FIGURE 2. Overall infection with blood parasites was marginally more prevalent in Song Sparrows (*Melospiza melodia*) from western Washington than birds from southern California (P=0.088 overall), with *Parahaemoproteus*, but not *Plasmodium*, significantly more prevalent in Washington (A). Among birds from Washington, where variation was sufficient for within-population tests, helminth burden (B) did not predict the probability of infection with blood parasites (P=0.484).

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FIGURE 1. Overall burdens of gut helminths were higher in Song Sparrows (*Melospiza melodia*) from western Washington than in sparrows from southern California. Although burdens of Acanthocephala were not different between populations (A), burdens of cestodes (B) and nematodes (C) were higher in Washington birds, leading to an overall higher helminth burden (D). Lines show population medians; statistics reflect Wilcoxon tests. Points were jittered randomly along the horizontal axis to avoid overlap and visualize all data more clearly.

deposited into the MalAvi database, including *Plasmodium* lineages PADOM09, BT7, and SIAMEX02 and *Parahaemoproteus* lineages PHEMEL02, ICTLEU01, PACDEC02, and JUHYE03. Overall, birds from Washington showed a nonsignificant trend toward higher probability of infection with any blood parasite (GLM, population_Washington parameter estimate=0.97, SE=0.58, $z_{1,53}$=1.66, P=0.103). The probability of infection with *Plasmodium* trended to be lower among Washington birds (GLM, population_Washington parameter estimate=–1.52, SE=0.86, $z_{1,53}$=–1.76, P=0.083). However, a significantly higher number of birds were infected with *Parahaemoproteus* in Washington (χ^2= 12.81, P< 0.001), because this parasite was not found in samples from California (Fig. 2A). Neither body condition nor its interaction with population predicted the probability of blood parasite infection (both P>0.362) and were thus removed from final models.

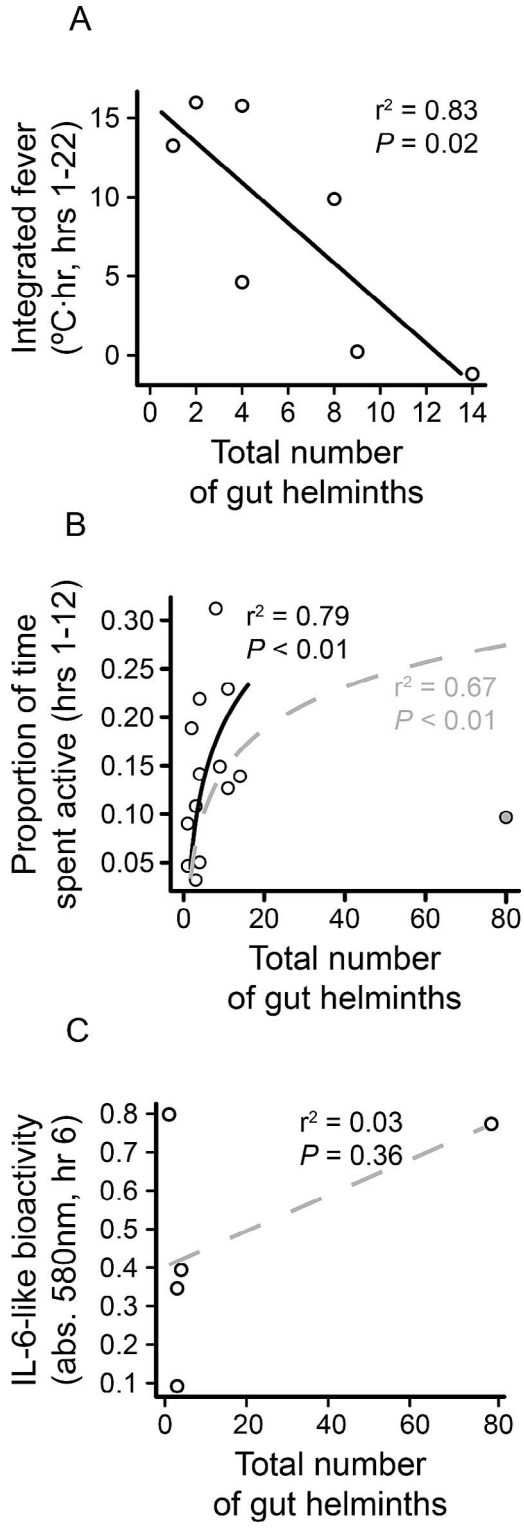
Within-population correlations

Because California birds showed very little variation in worm burdens, whereas Washington birds exhibited substantial variation, we chose to examine relationships with helminth burden at the individual level for Washington animals only. Among Washington birds, the probability of infection with malaria parasites did not vary with helminth burdens (Fig. 2B; GLM, total number of worms parameter estimate=–0.025, SE=0.035, $t_{1,20}$=–0.71, P=0.484). Results were similar when considering only *Parahaemoproteus*, the more prevalent malaria parasite in Washington (GLM, total number of worms parameter estimate=–0.019, SE=0.034, $t_{1,20}$=–0.55, P=0.593). Patterns of infection with blood parasites were qualitatively identical when excluding an outlier with 80 gut helminths (Fig. 1). In contrast, fever was less pronounced in individuals with higher numbers of gut helminths and more pronounced in individuals with higher body condition (Fig. 3A; GLM, total number of worms parameter estimate=–1.10, SE=0.27, $t_{1,4}$=–4.08, P=0.015; body condition parameter estimate=2.14, SE=0.75, $t_{1,4}$=2.89, P=0.046; adjusted r^2=0.83). Similarly, individuals with higher worm burdens showed less lethargy after LPS injection (Fig. 3B, black; GLM, natural log of total number of helminths+1 parameter estimate=0.08, SE=0.01, $t_{1,12}$=7.15, P<0.001, r^2=0.79). When an outlier was included in the analysis of lethargy, this pattern was less pronounced (Fig. 3B, gray; GLM, natural log of total number of helminths+1 parameter estimate=0.06, SE=0.01, $t_{1,13}$=5.37, P<0.001, adjusted r^2=0.67). However, IL-6–like bioactivity showed no trend with gut helminth burden, although the sample size for this metric was considerably smaller (Fig. 3C; parameter estimate=0.005, SE=0.004, $t_{1,3}$=1.07, P=0.36, adjusted r^2=0.035). In all within-population analyses, except that of fever (see above), neither body condition nor its interaction with total worm burden predicted the dependent variable (all P>0.300) and were thus removed from final models.

DISCUSSION

We found that Song Sparrows with higher gut helminth burdens exhibited less pronounced immune APRs to simulated bacterial infection (inoculation with LPS) at both the individual and population levels. We also show a nonsignificant trend of higher blood parasite prevalence in birds from the population with higher levels of gut helminth infection (western Washington). This is the first study to show an association between gut-helminth parasitism and variability in immune responses among songbird individuals, both within a single population and between different populations.

At the population level, birds in Washington had higher rates of helminth infection, higher helminth burdens among infected animals (Fig. 1B–D), and less severe APRs than birds from California (Adelman et al. 2010a). Although this pattern is consistent with a role for helminth infection in immune variability, myriad other factors differ among


A
Integrated fever (ºC·hr, hrs 1-22)
r² = 0.83
P = 0.02
Total number of gut helminths
B
Proportion of time spent active (hrs 1-12)
r² = 0.79
P < 0.01
r² = 0.67
P < 0.01
Total number of gut helminths
C
IL-6-like bioactivity (abs. 580nm, hr 6)
r² = 0.03
P = 0.36
Total number of gut helminths

these sites, including temperature, precipitation, and length of breeding season, any of which could potentially drive differences in immune function (Johnston 1954; National Oceanic and Atmospheric Administration 2018). Therefore, we performed an intrapopulation analysis to better control for those factors. Rates of helminth infection in California birds were so low that variation in infection intensity was insufficient to test for within-population associations between helminths and the APR. In Washington birds, however, nearly all birds were infected with helminths, with considerable variation among individuals. Because helminth parasites tend to aggregate, with most hosts showing very low burdens and a few hosts showing very high burdens, encountering a single Washington bird with 80 helminths was unsurprising (Shaw et al. 1998). Among Washington birds, we found a negative association between helminth burden and the severity of fever and sickness behaviors (Fig. 3A, B), similar to our between-population findings. Because our analysis excluded the only bird from the study that appeared to be fighting an active bacterial infection (as determined by markedly elevated levels of IL-6–like bioactivity), the trends observed are unlikely to result from concurrent bacterial infections. Despite the small sample size, the trend observed in Washington birds suggested that helminth burden could explain some of the variation in APR seen in Song Sparrows, consistent with prior work in mammals (Maizels et al. 2004). Experimental manipulations, however, will be required to confirm this relationship.

We predicted that a reduced APR, associated with increased helminth parasitism, would leave birds more susceptible to malaria infection. However, we did not find such a relationship, either between populations or within the Washington population. Although these findings run counter to our predictions, they are not without precedent. For example, in mice experimentally coinfected with the malarial parasite *Plasmodium chabaudi* and the filarial nematode *Nippostrongylus brasiliensis*, nematodes exerted immunosuppressive effects. However, these effects did not alter levels of malaria infection (Griffiths et al. 2015), which suggests that although helminths may modulate inflammatory immune responses generally, the specific responses may not affect defenses against blood parasites.

Coinfection with multiple micro- and macroparasites is usually the natural state of animals in the wild but is difficult to study and is seldom described (Petney and Andrews 1998). Understanding how such coinfections alter immune responses will be critical in revealing their effects on pathogen transmission (Ezenwa and Jolles 2011). Our data, in combination with recent work in wild rodents and large mammals, suggest that helminth infection could affect responses to microparasite infection in diverse wild animals (Ezenwa 2016). However, more studies that experimentally manipulate helminth burdens in the wild will be necessary to truly understand these relationships.

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FIGURE 3. Among Song Sparrows (*Melospiza melodia*) from western Washington, burdens of gut helminths predicted the severity of febrile and behavioral responses after injection with lipopolysaccharide (LPS), which simulates bacterial infection. The duration and magnitude of fever correlated negatively with helminth loads (A), whereas overall locomotor activity was higher in birds with higher helminth burdens (B). In contrast, inflammatory cytokine signaling, as measured by interleukin-6–like (IL-6–like) bioactivity (C), did not correlate with gut helminth load. Lines show predictions from general linear models. In panel B, the gray dashed line indicates model predictions when a single outlier (gray-filled circle) is included in the analysis.

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