Plasmodium prevalence across avian host species is positively associated with exposure to mosquito vectors

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SUMMARY

The prevalence of vector-borne parasites varies greatly across host species, and this heterogeneity has been used to relate infectious disease susceptibility to host species traits. However, a few empirical studies have directly associated vector-borne parasite prevalence with exposure to vectors across hosts. Here, we use DNA sequencing of blood meals to estimate utilization of different avian host species by *Culex* mosquitoes, and relate utilization by these malaria vectors to avian *Plasmodium* prevalence. We found that avian host species that are highly utilized as hosts by avian malaria vectors are significantly more likely to have *Plasmodium* infections. However, the effect was not consistent among individual *Plasmodium* taxa. Exposure to vector bites may therefore influence the relative number of all avian *Plasmodium* infections among host species, while other processes, such as parasite competition and host-parasite coevolution, delimit the host distributions of individual *Plasmodium* species. We demonstrate that links between avian malaria susceptibility and host traits can be conditioned by patterns of exposure to vectors. Linking vector utilization rates to host traits may be a key area of future research to understand mechanisms that produce variation in the prevalence of vector-borne pathogens among host species.

Key words: Avian malaria parasites, malaria vectors, host–vector interactions, mosquito feeding patterns, *Plasmodium*.

INTRODUCTION

Host exposure and susceptibility to a pathogen are fundamental determinants of infection (Poulin, 2011). Infection dynamics driven by differential exposure and susceptibility among host individuals may manifest as variation in prevalence, which represents the proportion of infected individuals in a host population. The prevalence of infection may be influenced by ecological attributes of a host species, and biologists have used these relationships to test predictions that relate pathogen pressure to host evolutionary ecology. For instance, infectious disease prevalence of different host species has been linked to development rates (Ricklefs, 1992; Tella *et al*. 1999), behaviour and sociality (Nunn *et al*. 2000; Nunn and Heymann, 2005; Fecchio *et al*. 2011, 2013; Krebs *et al*. 2014; Lutz *et al*. 2015), colouration and ornamentation (Hamilton and Zuk, 1982; Scheuerlein and Ricklefs, 2004) and habitat use (Mendes *et al*. 2005; Krama *et al*. 2015).

Avian Haemosporida comprise a suitable system to explore the relationships between host species traits and infection rates, owing to the substantial variation in prevalence among host species (Fallon *et al*. 2003*a*, 2005; Latta and Ricklefs, 2010), even within the same ecological community (Ricklefs *et al*. 2005; Svensson-Coelho *et al*. 2013). Exposure to avian Haemosporida is mediated by biting dipteran vectors that transmit the parasites between hosts. The taxonomic family of competent dipteran vectors varies among parasites (Valkiūnas, 2005). For example, *Haemoproteus* parasites are vectored by biting midges (Ceratopogonidae), while avian *Plasmodium* is transmitted by mosquitoes (Culicidae).

Variation in haemosporidian prevalence among avian host species has been linked to numerous host traits. For instance, Hamilton and Zuk (1982) suggested that bright plumage and elaborate secondary sexual characters evolved under parasite-mediated sexual selection. Here, costly plumage and displays demonstrate individual resistance among highly parasitized host species, thus driving a positive association between haemosporidian prevalence and plumage coloration. In addition, Ricklefs (1992) proposed that the prolonged embryonic development periods of some host species permit the development of more competent immune systems, thus lowering infection rates of blood parasites including Haemosporida. Ecological attributes of host species have also been assumed to influence exposure rates to suitable vectors, driving a positive correlation with haemosporidian prevalence. Association with

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habitats that may harbour more vectors is related to higher haemosporidian prevalence among host species (van Riper *et al*. 1986; Super and van Riper, 1995; Tella *et al*. 1999; Mendes *et al*. 2005; González *et al*. 2014; Krama *et al*. 2015). Larger body size may be related to increased prevalence (Scheuerlein and Ricklefs, 2004), potentially because larger bodied birds attract more vectors. In addition, nesting traits such as nest height in the canopy and nest type may also mediate exposure to vectors (Fecchio *et al*. 2011; González *et al*. 2014; Lutz *et al*. 2015).

Apparent relationships between host traits and haemosporidian prevalence may be confounded, leading to uncertainty over the mechanisms that drive these correlations. For instance, studies relating parasite prevalence to host traits associated with susceptibility often fail to control directly for variation in exposure to potentially infectious vectors (Ricklefs, 1992; Tella *et al*. 1999). Other studies have focused on host traits assumed to influence exposure to a parasite vector, but fail to directly link those host traits to variation in contacts with vectors (Scheuerlein and Ricklefs, 2004; Mendes *et al*. 2005; Fecchio *et al*. 2011, 2013; Krama *et al*. 2015; Lutz *et al*. 2015). The paucity of studies that explore patterns of *Plasmodium* prevalence with respect to variation in host–vector interactions is particularly concerning since some avian *Plasmodium* vectors are known to obtain blood meals at rates disproportionate to the host's relative abundance (Kilpatrick *et al*. 2006; Hamer *et al*. 2009). Over-utilized hosts (more vector encounters than predicted by relative abundance) may receive more vector bites than under-utilized species, thus providing more opportunities for infection and greater selective pressures on parasites to evolve mechanisms to surmount specialized defences of these particular hosts.

A few studies have related the distribution of haemosporidian parasites directly to host–vector encounter rates (Gager *et al*. 2008; Hellgren *et al*. 2008; Medeiros *et al*. 2013; Carlson *et al*. 2015). Both Gager *et al*. (2008) and Medeiros *et al*. (2013) suggested that associations with different vector species do not modulate the distribution of *Plasmodium* species among avian host species. Rather, host compatibility mechanisms involving differential susceptibility to various putative parasite species likely play a larger role. However, neither of these studies focused explicitly on *Plasmodium* prevalence, but instead compared the relative similarity of *Plasmodium* assemblages amongst host species (Medeiros *et al*. 2013) and between host species and vector species (Gager *et al*. 2008; Medeiros *et al*. 2013). Here, we analyse patterns of host utilization by known *Plasmodium* vectors and relate these patterns explicitly to variation in avian *Plasmodium* prevalence among a community of avian host species. We find that vector-biting rates are associated with the overall prevalence of *Plasmodium* parasites in host species, but that individual parasite lineages exhibit a variety of host distribution patterns that likely reflect specialized, coevolved host–pathogen interactions.

METHODS

We studied the relationship between host utilization by vectors and *Plasmodium* prevalence among a community of birds in suburban Chicago, IL, USA. The study site included 17 separate sampling locations within residential areas, city parks and gardens, and urban wilderness areas (characterized by small patches of natural habitat surrounded by an urban matrix). The site is inhabited by a bird community typical of urban landscapes of the Midwestern region of the USA. Previous work has indicated that ornithophilic *Culex* mosquitoes are the primary vectors of avian mosquito-borne pathogens in the area (Hamer *et al*. 2009), including *Plasmodium* parasites (Medeiros *et al*. 2013). Moreover, *Culex* species may vector similar suites of avian *Plasmodium* taxa (Valkiūnas, 2005; Kimura *et al*. 2010). *Culex pipiens* represents roughly 95% of the ornithophilic mosquitoes on the site, with *Culex restuans* accounting for 5% (Chaves *et al*. 2011). *Culex salinarius* was rare (<1% of blood meals), but present in our sample, as well.

Resident birds were captured in mist nets throughout the study site during 2006 and 2007 from May to September. Blood (generally <50 μL) was obtained and stored at −20 °C for later analysis. DNA from the blood was extracted with a 5 M ammonium acetate solution, and purified through a standard alcohol precipitation. We used polymerase chain reaction (PCR) and DNA sequencing to diagnose Haemosporida infections and assign putative parasite species (see Fallon *et al*. 2003*b*; Ricklefs *et al*. 2005; Fecchio *et al*. 2013; Svensson-Coehlo *et al*. 2013 for more details). Bird surveys (point counts modelled with distance sampling) were conducted across 30 nearby sites in 2010 to estimate host species abundances. Surveys were conducted from approximately 15 min before sunrise to 2 h after sunrise during June–September. Abundances were estimated in the package distance in program R. Key functions were selected based on Akaike information criteria and goodness of fit tests. Individual species densities were estimated when ⩾30 detection events were recorded. For species with fewer than 30 detections, we estimated a detection function for similar species that fell within a group of birds of similar size and behaviour. For example, small arboreal passerines such as New-World warblers (family Parulidae) and black-capped chickadees (*Poecile atricapillus*, Paridae) were pooled, as were tree and barn swallows (*Tachycineta bicolor, Hirundo rustica*, respectively).

The density of each species was estimated as the density of all birds in the group multiplied by the proportion of that species within that group. Density estimates were similar to those of surveys conducted in 2005 and 2006 in the same general area (Loss *et al*. 2009).

Blooded mosquitoes were sampled from the same or nearby sites during 2005–2012 with standard centers of disease control and prevention (CDC) light traps, CDC gravid traps and backpack aspirators (Hamer *et al*. 2009). Mosquitoes were sexed, identified to the species level, and stored at −80 °C for later processing. The vertebrate sources of blood meals from blood-fed mosquitoes were determined through molecular protocols that included DNA extraction, selective amplification of vertebrate DNA through PCR and sequencing of the amplicon. The blood meal analysis for samples collected between 2005 and 2008 followed previously published protocols involving PCR targeting the vertebrate cytochrome *b* (cyt *b*) gene (Hamer *et al*. 2009). For blood-fed mosquitoes collected between 2009 and 2012, we used a modified approach that involved PCR primers for the vertebrate cytochrome c oxidase 1 (COI) and cyt *b* gene. Briefly, we initially screened extracted DNA from the blooded abdomen with the vertebrate primer cocktail PCR targeting a 648 base pair (bp) region of the cytochrome c oxidase 1 (COI) gene utilizing the Epicenter Failsafe PCR kit (Epicentre, Madison, Wisconsin) (Ivanova *et al*. 2007; Kent, 2009*a*). Forward primers VF1_t1, VF1d_t1 and VF1i_t1 and reverse primers VR1d_t1, VR1_t1 and VR1i_t1 were each mixed at a ratio of 1:1:2 (Ivanova *et al*. 2007; Kent *et al*. 2009*b*). Thermo-cycling conditions were as follows: 94 °C for 1 min, five cycles of 94 °C for 30 s, 50 °C for 40 s, and 72 °C for 1 min, followed by 35 cycles of 94 °C for 30 s, 54 °C for 40 s, and 72 °C for 1 min, with a final extension at 72 °C for 10 min (Ivanova *et al*. 2007). PCR products were visualized on a 2% agarose gel and samples producing a 648 bp amplicon were purified with ExoSAP-IT for PCR product clean-up (Affymetrix USB, Cleveland, Ohio) before sequencing. Following the sequencing of the COI amplicon, we either accepted the result ending the blood meal analysis, or we continued with a subsequent PCR targeting a different gene. Criteria for continued PCR-sequencing included (1) no PCR amplicon, (2) poor sequence quality, (3) evidence of mixed DNA (double-nucleotide peaks in chromatograph), and (4) human basic local alignment search tool (BLAST) match. If the results of the vertebrate cocktail PCR yielded one of these four outcomes, we continued with a second blood meal PCR targeting a 358 bp region of the cyt *b* gene (Boakye *et al*. 1999; Hamer *et al*. 2009). Then, based on the same decision criteria, we either accepted the PCR results and finished the analysis, or continued with a third and final PCR ('herp' primers) targeting a 228 bp region of the cyt *b* gene (Cupp *et al*. 2004; Hamer *et al*. 2009). The hierarchical use of two genes and two different cyt *b* primer sets was done to maximize efficiency and minimize the number of unidentified blood meals, as primer efficacy can vary between vertebrate taxonomy. In addition, sequentially smaller amplicon targeted by the cyt *b* primers aided in identifying more digested blood meals that had greater DNA degradation. All blooded *Culex* mosquitoes (including *Cx. pipiens, Cx. restuans, Cx. salinarius*) were differentiated molecularly based on an established PCR technique using the same template DNA used in the blood meal analysis (Crabtree *et al*. 1995).

To analyse whether utilization by *Culex* vectors was random across avian hosts, we used *rmultinom* from the stats package in program R to simulate the total number of avian blood meals collected from 2005 to 2012 ($N = 1221$) and construct random distributions of the expected number of blood meals based on relative host abundance estimated from the point-count surveys. Simulations were repeated 100 000 times across the 32 bird species included in this analysis, from which we extracted the resulting 99% confidence intervals of the expected number of blood meals for each host species.

We estimated vector utilization in three, related ways: (1) the $\log_{10}$-transformed total number of *Culex* blood meals observed from 2005 to 2012; (2) the residual number of *Culex* blood meals after regressing the $\log_{10}$-transformed total number of blood meals against the $\log_{10}$-transformed host density; (3) a host selection ratio (proportion of blood meals from species *i* divided by the proportion of species *i* in the avian community), which is a common metric in many mosquito-feeding studies (Kilpatrick *et al*. 2006; Hamer *et al*. 2009). Bird species that were detected in blood meals, but were not recorded during bird surveys, were given a default density of 0·01 individuals per hectare, equal to the least dense recorded species in the analysis. We added 1 to all counts of blood meals before $\log_{10}$-transformation of the data. We used a generalized linear model in R, assuming a quasibinomial error distribution, to test the effect of vector utilization on *Plasmodium* prevalence. We used the cbind function to input the number of infected and uninfected individuals for each host species in the analysis. Models were weighted by the $\log_{10}$ of the sample size for each species. In the text, effect estimates for various models are denoted by 'β'. We included 32 summer-resident avian host species that had at least two blood samples. These results were similar to more restrictive analyses that included summer-resident host species with 10 blood samples or more. Birds that migrate through the site but do not breed were not included in any analysis.

All fieldwork was conducted with the permission from the Illinois Department of Works. Bird

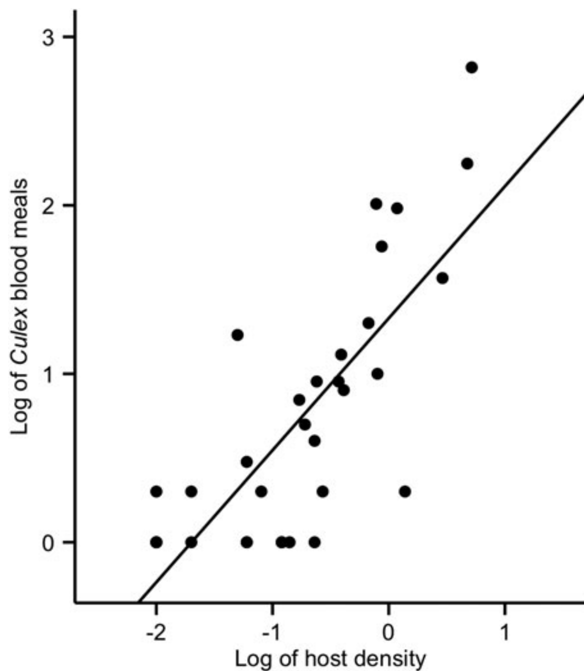

Fig. 1. Relationship between *Culex* blood meals and host density. Both blood meals and host density are $\log_{10}$-transformed.

sampling was conducted under approvals for animal use from the University of Illinois Animal Use Protocol no. 03034 and Institutional Animal Care and Use Committee at Michigan State University, Animal Use Form no. 12/03-152-00 and conformed to generally accepted standards of animal use.

RESULTS

A total of 1221 identified *Culex* blood meals were derived from avian hosts included in this analysis. The number of *Culex* blood meals varied among avian hosts, from 0 (several species) to 658 (54% of the total) from American robins (*Turdus migratorius*). *Culex* blood meals from a particular host were directly related to the host density (linear regression, $\beta = 0{\cdot}78$, $P < 0{\cdot}001$, $R^2 = 0{\cdot}60$; Fig. 1).

Random feeding by the *Culex* vectors of *Plasmodium*, which would result in an encounter rate proportional to the host's relative density, could be rejected for several summer-resident host species. We obtained more blood meals from American robin, blue jay (*Cyanocitta cristata*), mourning dove (*Zenaida macroura*), and northern cardinal (*Cardinalis cardinalis*) than expected by chance, and fewer blood meals from American goldfinch (*Carduelis tristis*), barn swallow, chipping sparrow (*Spizella passer*ina), common grackle (*Quiscalus quiscula*), downy woodpecker (*Picoides pubescens*), European starling (*Sturnus vulgaris*), grey catbird (*Dumetella carolinensis*), house sparrow (*Passer domesticus*), indigo bunting (*Passerina cyanea*), red-winged blackbird (*Agelaius phoeniceus*), song sparrow (*Melospiza melodia*), warbling vireo (*Vireo gilvus*), and yellow warbler (*Setophaga petechia*). We could not reject an encounter rate proportional to abundance for other species in the analysis, which included the relatively common black-capped chickadee, brown-headed cowbird (*Molothrus ater*), cedar waxwing (*Bombycilla cedrorum*), and house finch (*Haemorhous mexicanus*). Results of the feeding simulations are summarized in Appendix Table A1, along with the quantitative measures of vector utilization used below.

Overall, 1887 individuals of 32 avian host species were screened for malaria parasites. *Plasmodium* prevalence varied strongly among species in the analysis (logistic regression, $P < 0{\cdot}001$), ranging from 0 to a high of 0·51 in American robins (Appendix Table A2). Prevalence of *Plasmodium* parasites across all host species included in this analysis was positively related to both $\log_{10}$-transformed total *Culex* blood meals ($\beta = 1{\cdot}0$, $P < 0{\cdot}001$; Fig. 2), residual $\log_{10}$-transformed *Culex* blood meals ($\beta = 1{\cdot}8$, $P < 0{\cdot}001$; Fig. 2), and the host selection ratio ($\beta = 0{\cdot}88$, $P < 0{\cdot}001$; Fig. 2), indicating that hosts that are fed upon more often by *Culex* mosquito vectors have a higher probability of infection with avian malaria parasites. Prevalence of *Plasmodium elongatum* and *Plasmodium cathemerium*, two well-sampled generalist parasites, was not significantly associated with the $\log_{10}$-transformed total *Culex* blood meals ($\beta_{P.elon} = -0{\cdot}18$, $P = 0{\cdot}53$; $\beta_{P.cath} = 0{\cdot}03$, $P = 0{\cdot}87$), residual $\log_{10}$-transformed *Culex* blood meals ($\beta_{P.elon} = 0{\cdot}25$, $P = 0{\cdot}57$; $\beta_{P.cath} = 0{\cdot}18$, $P = 0{\cdot}53$), or the selection ratio ($\beta_{P.elon} = 0{\cdot}14$, $P = 0{\cdot}60$, $\beta_{P.cath} = -0{\cdot}11$, $P = 0{\cdot}55$; Fig. 3).

Plasmodium parasites did not occur in the non-passerine hosts in our sample, including the downy woodpecker ($N = 16$), northern flicker ($N = 5$) and the mourning dove ($N = 27$), despite the last species being over-utilized by local *Plasmodium* vectors for blood meals.

DISCUSSION

Our results demonstrate a general association between exposure to mosquitoes and *Plasmodium* prevalence in an assemblage of avian hosts. *Plasmodium* prevalence across host species was correlated with the number of vector blood meals that were derived from that species. The number of vector blood meals is related to the likelihood of a vector-borne pathogen encountering a particular host species, and this may drive the association between total blood meals and *Plasmodium* prevalence. Indeed, if mosquito-host relationships are stable over evolutionary timescales, an increase in encounters with specific host species may provide strong selective pressures on *Plasmodium* parasites to evolve mechanisms to circumvent specialized host defences.

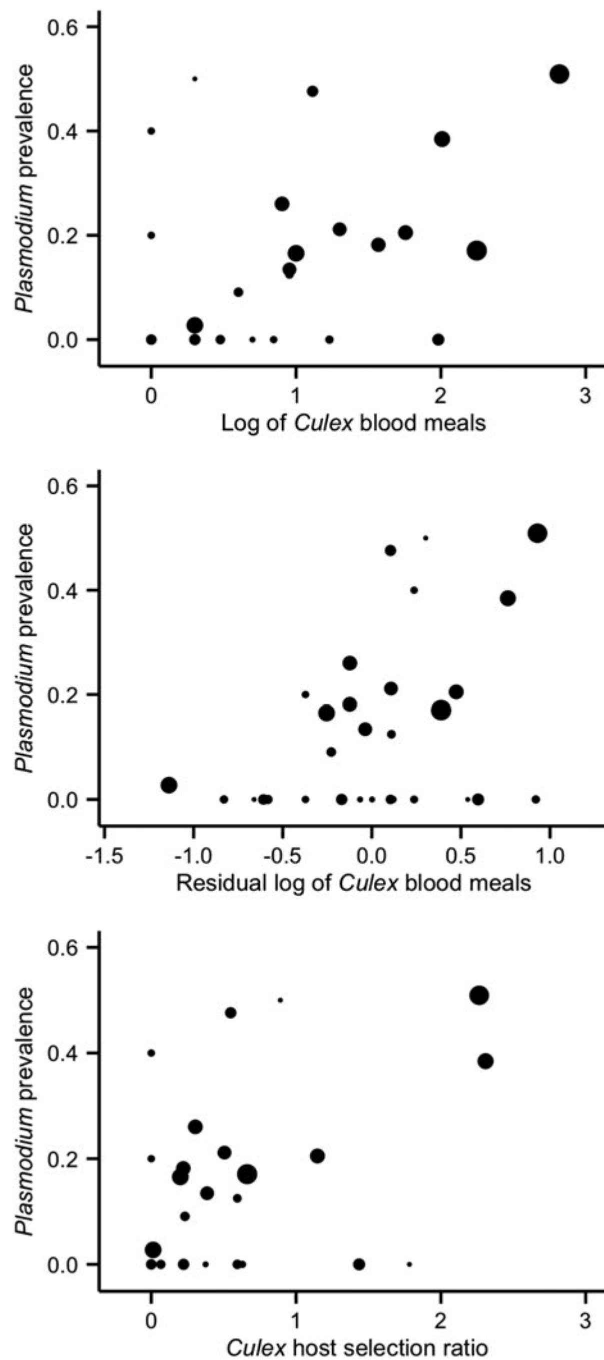

Fig. 2. Relationship between total *Plasmodium* prevalence and total *Culex* blood meals (top), residual *Culex* blood meals regressed against host density (middle), and the host selection ratio (bottom). Total *Culex* blood meals are $\log_{10}$-transformed. Blue Jays, with an outlying selection ratio of 5·7, are not portrayed on the host selection ratio plot. Dot size is proportional to host sample size for prevalence and thus, weight in the logistic regression models.

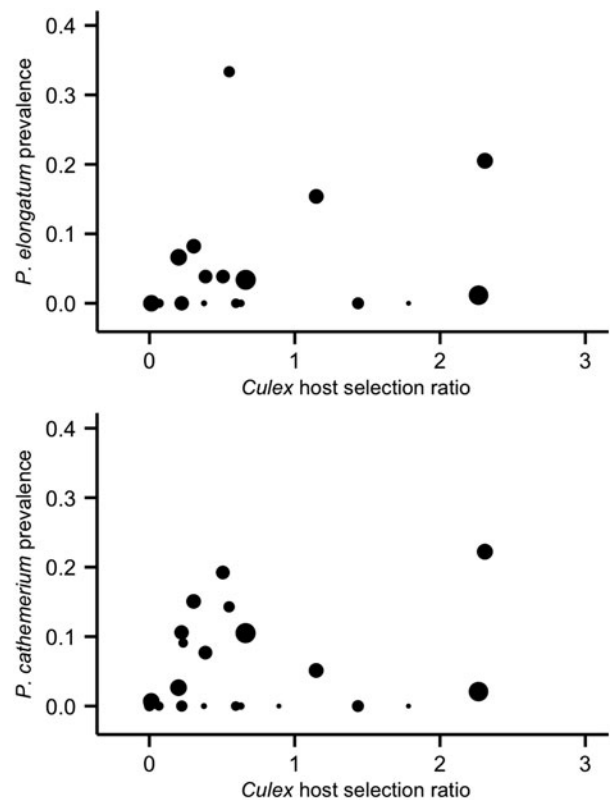

Fig. 3. Relationship between the host selection ratio and *Plasmodium elongatum* (top) and *Plasmodium cathemerium* (bottom). Blue Jays, with outlying selection ratio of 5·7, are not portrayed on the plots. Dot size is proportional to host sample size for prevalence and thus, weight in the logistic regression models.

Plasmodium prevalence was also correlated with the residuals of vector blood meals after regressing vector blood meals against host density and the host selection ratio. Both these metrics control for abundance, and may be proportional to the average number of mosquito bites an individual of a particular host species receives. Simple theoretical models of multi-pathogen vector-borne disease dynamics (such as the Ross–Macdonald model [Ross, 1911; Macdonald, 1957]) and several empirical investigations (Snow *et al*. 1988; Nevill *et al*. 1996; Charlwood *et al*. 1998; Martínez-de la Puente *et al*. 2013) suggest that these associations between prevalence and the frequency of blood meals relative to host abundance may arise from an increase in infection probability with more vector encounters. Alternatively, the association may result from mosquitoes being attracted to individual birds that are infected with malaria. In one experimental study, *Cx. pipiens* were more attracted to canaries that had chronic *Plasmodium relictum* infections (Cornet *et al*. 2013). If this effect occurred broadly across different host and *Plasmodium* species in the wild, *Plasmodium* parasites might alter vector-feeding patterns and the transmission of many mosquito-borne zoonotic diseases. However, another study (Lalubin *et al*. 2012) showed *Cx. pipiens* were less attracted to wild great tits (*Parus major*) with naturally acquired *Plasmodium* infections, calling the generality of these patterns into question. Further research is necessary to discriminate between these alternate hypotheses.

Our results are consistent with the level of overall *Plasmodium* prevalence being related to vector utilization, with processes including parasite–parasite competition and host–parasite coevolution determining more restricted host breadths of individual parasite species. We identified 17 putative avian *Plasmodium* species based on cyt *b* lineages. The distributions of these putative *Plasmodium* species vary starkly across hosts within this community (Medeiros *et al*. 2013, 2014). Previous analyses suggested that host compatibility plays a larger role in delimiting the host ranges of individual avian *Plasmodium* taxa than a mosquito-imposed encounter rate (Medeiros *et al*. 2013). Given constraints of sample sizes and the distributions across host species for individual lineages, we did not directly relate the prevalence of all individual lineages to vector-utilization estimates. However, the prevalence of two well-sampled, generalized *Plasmodium* parasites varied independently of vector utilization across local hosts. This may be associated with the scarcity of these parasites on American robins (Medeiros *et al*. 2013), which are the most frequent source of avian blood meals in the area, and which are generally over-utilized by blood-seeking *Culex* mosquitoes in this study site (Hamer *et al*. 2009) and elsewhere (Kilpatrick *et al*. 2006) relative to their abundance. While American robins maintain a low prevalence of generalized *Plasmodium* parasites despite being over-utilized by *Plasmodium* vectors, they are infected locally with up to five apparently specialized *Plasmodium* species (Medeiros *et al*. 2014). Three of these specialized *Plasmodium* species occur at relatively high prevalence within this robin population (Medeiros *et al*. 2013, 2014). Robins, with their relatively high-density and vector-mediated encounter rate, likely represent a stable resource for avian *Plasmodium*, promoting the evolution of specialization. Specialized parasites may be more proficient than generalists on shared host species (Medeiros *et al*. 2014), potentially outcompeting generalists on local robins.

Our results, question the practice of relating avian malaria prevalence to species traits without controlling for differences in vector exposure. Mosquitoes feed heterogeneously across host species, and our analysis suggests that this may influence the probability of *Plasmodium* infection. Host traits such as sociality (Fecchio *et al*. 2013), habitat preference (Tella *et al*. 1999; Mendes *et al*. 2005), night roosting behaviours (Garvin and Remsen, 1997), nesting characteristics (González *et al*. 2014; Lutz *et al*. 2015) and body size (Scheuerlein and Ricklefs, 2004) are often assumed to influence exposure to parasite vectors and the prevalence of vector-transmitted pathogens. Additional studies relating these traits directly to vector-utilization patterns while integrating vector prevalence would improve our understanding of the ecological drivers of variation in infection rates of vector-borne pathogens across host species.

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APPENDIX

Table A1. Estimates of vector utilization

Scientific name	Four-letter code	Blood meal confidence limit 0·005%	Blood meal confidence limit 0·995%	Total *Culex* blood meals	Residual blood meals	Host selection ratio	Estimated individuals ha^{-1}
Geothlypis trichas	COYE	0	3	0	0·24	0·00	0·01
Pipilo erythrophthalmus	EATO	0	3	1	0·54	1·78	0·01
Myiarchus crinitus	GCFL	0	3	0	0·24	0·00	0·01
Setophaga ruticilla	AMRE	0	3	0	0·24	0·00	0·01
Tachycineta bicolor	TRES	0	5	0	0·00	0·00	0·02
Toxostoma rufum	BRTH	0	5	1	0·30	0·89	0·02
Cyanocitta cristata	BLJA	0	8	16	0·92	5·71	0·05
Vireo olivaceus	REVI	0	9	0	−0·37	0·00	0·06
Icterus galbula	BAOR	0	9	2	0·10	0·59	0·06
Colaptes auratus	YSFL	0	9	0	−0·37	0·00	0·06
Empidonax traillii	WIFL	0	11	1	−0·17	0·22	0·08
Picoides pubescens	DOWO	1	14	0	−0·61	0·00	0·12
Passerina cyanea	INBU	1	14	0	−0·61	0·00	0·12
Hirundo rustica	BARS	2	16	0	−0·66	0·00	0·14
Poecile atricapillus	BCCH	3	18	6	0·12	0·63	0·17
Troglodytes aedon	HOWR	3	20	4	−0·07	0·38	0·19
Spizella passerina	CHSP	5	23	3	−0·23	0·23	0·23
Setophaga petechia	YWAR	5	23	0	−0·83	0·00	0·23
Bombycilla cedrorum	CEDW	5	24	8	0·11	0·59	0·24
Vireo gilvus	WAVI	6	26	1	−0·58	0·07	0·27
Agelaius phoeniceus	RWBL	10	33	8	−0·04	0·39	0·37
Molothrus ater	BHCO	11	35	12	0·10	0·55	0·39
Melospiza melodia	SOSP	12	36	7	−0·12	0·30	0·41
Quiscalus quiscula	COGR	23	54	19	0·11	0·51	0·67
Cardinalis cardinalis	NOCA	28	61	101	0·76	2·31	0·78
Dumetella carolinensis	GRCA	29	63	9	−0·25	0·20	0·80
Haemorhous mexicanus	HOFI	32	67	56	0·47	1·15	0·87
Zenaida macroura	MODO	47	87	95	0·60	1·44	1·18
Carduelis tristis	AMGO	56	100	1	−1·14	0·01	1·38
Sturnus vulgaris	EUST	133	194	36	−0·12	0·22	2·90
Passer domesticus	HOSP	229	304	176	0·39	0·66	4·74
Turdus migratorius	AMRO	253	329	658	0·93	2·26	5·18

Table A2. *Plasmodium* prevalence

Scientific name	Four-letter code	Total samples	Total *Plasmodium* infections	Total *P. elongatum* infections	Total *P. cathemerium* infections
Geothlypis trichas	COYE	5	2	0	0
Pipilo erythrophthalmus	EATO	2	0	0	0
Myiarchus crinitus	GCFL	3	0	0	0
Setophaga ruticilla	AMRE	6	0	0	0
Tachycineta bicolor	TRES	3	0	0	0
Toxostoma rufum	BRTH	2	1	1	0
Cyanocitta cristata	BLJA	7	0	0	0
Vireo olivaceus	REVI	5	1	0	0
Icterus galbula	BAOR	11	0	0	0
Colaptes auratus	YSFL	5	0	0	0
Empidonax traillii	WIFL	21	0	0	0

Table A2. (*Cont.*)

Scientific name	Four-letter code	Total samples	Total *Plasmodium* infections	Total *P. elongatum* infections	Total *P. cathemerium* infections
Picoides pubescens	DOWO	16	0	0	0
Passerina cyanea	INBU	7	0	0	0
Hirundo rustica	BARS	2	0	0	0
Poecile atricapillus	BCCH	5	0	0	0
Troglodytes aedon	HOWR	3	0	0	0
Spizella passerina	CHSP	11	1	0	1
Setophaga petechia	YWAR	7	0	0	0
Bombycilla cedrorum	CEDW	8	1	0	0
Vireo gilvus	WAVI	10	0	0	0
Agelaius phoeniceus	RWBL	52	7	2	4
Molothrus ater	BHCO	21	10	7	3
Melospiza melodia	SOSP	73	19	6	11
Quiscalus quiscula	COGR	52	11	2	10
Cardinalis cardinalis	NOCA	117	45	24	26
Dumetella carolinensis	GRCA	151	25	10	4
Haemorhous mexicanus	HOFI	78	16	12	4
Zenaida macroura	MODO	27	0	0	0
Carduelis tristis	AMGO	146	4	0	1
Sturnus vulgaris	EUST	66	12	0	7
Passer domesticus	HOSP	533	91	18	56
Turdus migratorius	AMRO	432	220	5	9

Table A3. Species common names, scientific names and four-letter codes

Four-letter code	Common name	Scientific name
COYE	Common yellowthroat	*Geothlypis trichas*
EATO	Eastern towhee	*Pipilo erythrophthalmus*
GCFL	Great crested flycatcher	*Myiarchus crinitus*
AMRE	American redstart	*Setophaga ruticilla*
TRES	Tree swallow	*Tachycineta bicolor*
BRTH	Brown thrasher	*Toxostoma rufum*
BLJA	Blue jay	*Cyanocitta cristata*
REVI	Red-eyed vireo	*Vireo olivaceus*
BAOR	Baltimore oriole	*Icterus galbula*
YSFL	Yellow-shafted flicker	*Colaptes auratus*
WIFL	Willow flycatcher	*Empidonax traillii*
DOWO	Downy woodpecker	*Picoides pubescens*
INBU	Indigo bunting	*Passerina cyanea*
BARS	Barn swallow	*Hirundo rustica*
BCCH	Black-capped chickadee	*Poecile atricapillus*
HOWR	House wren	*Troglodytes aedon*
CHSP	Chipping sparrow	*Spizella passerina*
YWAR	Yellow warbler	*Setophaga petechia*
CEDW	Cedar waxwing	*Bombycilla cedrorum*
WAVI	Warbling vireo	*Vireo gilvus*
RWBL	Red-winged blackbird	*Agelaius phoeniceus*
BHCO	Brown-headed cowbird	*Molothrus ater*
SOSP	Song sparrow	*Melospiza melodia*
COGR	Common grackle	*Quiscalus quiscula*
NOCA	Northern cardinal	*Cardinalis cardinalis*
GRCA	Gray catbird	*Dumetella carolinensis*
HOFI	House finch	*Haemorhous mexicanus*
MODO	Mourning dove	*Zenaida macroura*
AMGO	American goldfinch	*Carduelis tristis*
EUST	European starling	*Sturnus vulgaris*
HOSP	House sparrow	*Passer domesticus*
AMRO	American robin	*Turdus migratorius*