Overlap in the Seasonal Infection Patterns of Avian Malaria Parasites and West Nile Virus in Vectors and Hosts

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Abstract. Multiple vector-borne pathogens often circulate in the same vector and host communities, and seasonal infection dynamics influence the potential for pathogen interactions. Here, we explore the seasonal infection patterns of avian malaria (Haemosporida) parasites (*Plasmodium* and *Haemoproteus*) and West Nile virus (WNV) in birds and mosquitoes in suburban Chicago. We show that both pathogens vary seasonally in *Culex* mosquitoes and avian hosts, but that patterns of covariation are complex. Different putative *Plasmodium* species varied asynchronously across the season in mosquitoes and birds, suggesting that different forces may govern their transmission. Infections of *Culex* mosquitoes with *Plasmodium* parasites were positively associated with WNV infections in pools of individuals aggregated from the same time and site, suggesting that these pathogens respond to common environmental drivers and co-circulate among the same host and vector populations. Future research should focus on these common drivers, and whether these pathogens interact in vectors and hosts.

INTRODUCTION

Numerous factors drive seasonal patterns of vector-borne pathogen transmission,¹ and understanding these processes increases our ability to predict when outbreaks are likely to occur.^{2,3} Some drivers of seasonal infection involve vector behavior and population dynamics. Seasonal shifts in vector utilization of hosts for blood meals have been demonstrated in numerous mosquito species and populations,^{4–9} and these shifts may influence the incidence of vector-borne infectious disease.^{10–12} Vector abundance and activity are associated with infection risk and are influenced by seasonal climate variation.^{13,14} Climate also influences seasonal changes in host behavior and physiology that affect pathogen transmission. Host reproduction is often seasonal and correlated with resource availability, which in turn may vary with weather and climate. Energetically expensive breeding activities may leave adults more susceptible to infection.^{15,16} In addition, host recruitment introduces immunologically naive juveniles, which increases the proportion of susceptible individuals in a population and promotes disease transmission.¹⁷ Finally, post-breeding dispersal and migration can influence contacts between hosts and vectors that affect disease transmission.^{18,19}

Co-circulation of pathogens (broadly defined here as the transmission of two or more pathogens in the same population at the same time) can have important implications for patterns of infection. Indeed, co-circulation is a critical factor permitting direct and indirect interactions among pathogens. Interactions between co-circulating pathogens can influence infection dynamics in both vertebrate hosts²⁰ and arthropod vectors.²¹ For instance, infection can change the susceptibility of vertebrates toward other pathogens,²² and simultaneous infection may have important implications for host physiology, morbidity, and mortality.²³ Simultaneous or sequential infection may even influence the competence of

vectors and affect pathogen development.^{24,25} Cumulatively, these effects can manifest at the population level and influence the transmission of pathogens within a host and vector community.²² Thus, pathogen co-circulation presents a mechanism by which non-zoonotic pathogens of wildlife may represent public health concerns by modulating zoonotic pathogen transmission.

Avian malaria parasites of the taxa *Plasmodium* and *Parahaemoproteus* (order: Haemosporida) are ubiquitous parasites that may co-circulate with zoonotic pathogens for which birds are reservoir hosts. These parasites have complex lifecycles that involve asexual reproduction in an avian host and sexual reproduction in a dipteran vector. *Parahaemoproteus* is vectored by *Culicoides* midges (Ceratopogonidae), whereas *Plasmodium* parasites are vectored by mosquitoes (Culicidae), including those of the genus *Culex*.²⁶ Avian *Plasmodium* infections within hosts can be highly dynamic. During the acute stage of an infection, parasitemia increases to relatively high levels, causing morbidity in the avian host.²⁶ If the host survives the acute infection, parasitemia in the blood often declines to lower levels. Low parasitemia in the blood generally persists through this chronic stage of infection,²⁷ and parasites may disappear from the blood stream, lying dormant in tissues. Relapses and recrudescence of low-level or dormant malaria infection may occur, especially during periods of host stress.^{26,28}

Previous studies have revealed seasonal patterns in haemosporidian prevalence,^{12,29} especially in temperate regions where variation in the annual climate cycle influences host and vector demography. A classic model of temperate avian malaria infection posits an age-structured bimodal peak in the seasonal *Plasmodium* prevalence among hosts.³⁰ The model suggests that malaria prevalence drops in winter as infection causes mortality in some hosts while others clear infections from the blood stream through host defense mechanism. Stress associated with reproduction drives a recrudescence of dormant infections among adult birds, elevating the prevalence.^{28,30} A second increase in prevalence is associated with the synchronous appearance of naive juveniles and large vector populations toward the end of the avian breeding season. However, empirical data do

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not always support this model. For instance, Cosgrove and others²⁹ showed that the expected seasonal pattern of malaria infection among blue tits (*Cyanistes caeruleus*) in Oxfordshire, United Kingdom, was absent in *Plasmodium relictum* and present only in hatch-year hosts for *Plasmodium circumflexum*.

West Nile virus (WNV) first appeared in North America in 1999.³¹ WNV is primarily maintained in an avian host–*Culex* mosquito vector transmission cycle. Occasionally, WNV is transmitted to other vertebrate hosts, including horses and humans, and can cause disease. In humans, most WNV infections produce mild symptoms, but occasionally infections may be severe, causing neurological impairment and even death.³² WNV has also been implicated in the decline of several North American bird populations,³³ and thus also represents a threat to avian conservation. As with Haemosporida, WNV transmission is seasonal throughout much of North America,³⁴ including the incidence of infection in humans, but with distinct annual and regional variability.^{7,11,35}

Here, we document seasonal infection patterns for these two common vector-borne pathogens in suburban Chicago, IL. A previous study identified a negative association between *Plasmodium* infection and WNV serostatus among avian hosts in this region.³⁶ The mechanisms for this negative association remain uncertain but one possibility is *Plasmodium*–WNV coinfection decreases host survivorship. An additional study from the same region demonstrated that *Culex* mosquitoes commonly ingest multiple hemoparasites while taking avian blood meals.³⁷ However, the seasonal infection dynamics of these common avian pathogens and their potential for broad co-circulation within a host and vector community remains inadequately described. Here, we use an extensive dataset on infections from both pathogens in *Culex* mosquitoes and avian hosts to quantify WNV and avian *Plasmodium* seasonal infection patterns and explore their co-circulation in hosts and vectors.

MATERIALS AND METHODS

Sample collection. The study was conducted at 17 sites in suburban Chicago, IL, during 2006 and 2007, from mid-May through mid-October.¹⁷ Generally, sampling was conducted at each of these sites bimonthly. Briefly, host-seeking *Culex* mosquitoes were caught in standard Centers for Disease Control and Prevention–style light traps baited with dry ice. Traps were set in the evening either at eye level (~1.5 m above the ground) or in the canopy (3–4 m above the ground), and collected in the morning of the following day. All captured mosquitoes were sexed, identified to the species level (with exception *Culex restuans* and *Culex pipiens*, which are morphologically indistinguishable in this population³⁸), and sorted based on collection site and date. Birds in this study were sampled in mist nets. Individual birds were identified to species, aged, and banded with a numbered aluminum band (U.S. Fish and Wildlife Service). A small (< 50 μ L) blood sample was also obtained. Only infection data from American robins (*Turdus migratorius*), house sparrows (*Passer domesticus*), northern cardinals (*Cardinalis cardinalis*), and house finches (*Haemorhous mexicanus*), four well-sampled avian hosts frequently bitten by mosquitoes,⁷ were included in analyses presented here. Avian host sampling was authorized by the appropriate permits including a Federal Bird

Banding Permit no. 06507, animal-use approvals 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.

Haemosporida infections were identified through established molecular methods. For avian samples, DNA was extracted from packed blood cells preserved in Longmire's lysis buffer and stored at –20°C until processing. Blood samples were digested with proteinase K for ~12 hours, and DNA was extracted with a 5 M ammonium acetate solution and purified by a standard alcohol precipitation. Initially, DNA samples were screened with a polymerase chain reaction (PCR) that targeted the haemosporidian *16S rRNA* gene.³⁹ Samples that screened positive were subjected to a second nested PCR that amplified a 552-base pair fragment of the cytochrome *b* gene.⁴⁰ The amplicon of this reaction was sequenced directly. Given that Haemosporida taxonomy is poorly resolved at the species level,⁴¹ putative species of haemosporidian parasites were assigned based on cytochrome *b* haplotype and host distribution, generally following guidelines in the work of Svensson-Coelho and others.⁴² The independent lineages of these parasites have been discussed in previous studies.^{36,43,44} We used avian serum from blood samples to test for the presence of WNV antibodies using an inhibition enzyme-linked immunosorbent assay (ELISA).^{17,45}

For *Culex* mosquitoes, DNA was extracted from pools of whole-bodied host-seeking female individuals that were collected from the same sites and at the same time. Extractions were carried out with Qiagen (Hilden, Germany) blood and tissue kits following the manufacturer's protocol. Pools that were screened for Haemosporida ranged from 1 to 36 (median = 15) individuals. *Culex pipiens* and *Cx. restuans* are not reliably distinguished morphologically in eastern North America. Therefore, pools represent a mixture of these species, although *Cx. pipiens* is generally more abundant throughout the study site.⁴⁶ *Culex* pools were screened for Haemosporida parasites following the same protocols described for avian blood samples. Because our data rely on samples of whole-bodied mosquitoes that naturally acquired *Plasmodium* parasites, we cannot differentiate between infected mosquito hosts and infectious mosquito vectors. Nevertheless, our data do suggest that these mosquitoes were infected with gametocytes during a previous vertebrate blood meal as all individuals pooled were carefully inspected for blood meals. While more carefully prepared mosquito samples (i.e., salivary gland extractions, mosquito salivation) would better inform the period when mosquitoes in this population would be infectious to avian hosts, our goal in this study is to focus on infection patterns in mosquitoes, especially as it relates to WNV transmission.

A portion of the pooled mosquito sample was used to test for WNV with a protocol detailed in the work of Loss and others.⁴⁷ Briefly, RNA was extracted from homogenized mosquito pools, and the extract was screened in a reverse transcriptase PCR with primers specific for the WNV envelope gene.⁴⁸ Pools screened for WNV ranged in size from 1 to 38 individuals (median = 25).

Tables that summarize sample sizes of mosquitoes and avian hosts across weeks during the transmission season are present in the Supplemental Material (Summary of Samples, Supplemental Tables 1–4).

Statistical analyses. We used general linear models and general linear mixed models, assuming various error distributions

depending on the nature of the dependent variable, to analyze seasonal WNV and Haemosporida infection dynamics. In general, we used Akaike information criteria corrected for small sample size (AICc) to select candidate models using the R package *bbmle* (Hamilton, Ontario, Canada). Each candidate set included a global model with all variables of interest including relevant interactions. Other models in the set were composed of the nested subsets of the global model, including a fixed intercept-only model. However, our nested model sets did not include interaction effects in absence of the main effects, or a squared quadratic term in the absence of a non-squared linear term. We assumed the model with the lowest AICc score was “best-fit” to the data. To aid in the direct comparison of seasonal variation among pathogens, we rely on predicted responses between week 20 and 40 (mid-May through early October) from best-fit statistical models. Throughout the article, this period is referred to as the transmission season. In the Supplemental Material (Statistical Analyses), we describe the details of specific analyses. In addition, we used predicted probabilities from the best-fit models to estimate minimum infection rates (MIRs; see Supplemental Material [Minimum Infection Rate Calculation] for equation and overall approach) for vectors with

the common *Plasmodium* lineages and WNV. Figures within this article were created with the R package *ggplot2*.⁴⁹

RESULTS

Mosquito infection with Haemosporida. We identified seven putative species of *Plasmodium* parasites from 170 infections in 377 *Culex* pools. We did not detect *Parahaemoproteus* spp., which are common avian Haemosporida typically vectored by *Culicoides*, in the mosquito pools, in contrast to results from other studies.^{50,51} A multinomial logistic regression model revealed seasonal effects on the *Plasmodium* infection status of *Culex* pools (Figure 1A). The best-fit model incorporated pool size and a quadratic week effect (weight = 0.60), but was similar in fit to a model that included year and interactions between year and both linear and quadratic week terms ($\Delta\text{AICc} = 1.9$, weight = 0.23; Supplemental Table 5). Models that included site of capture fit the data relatively poorly. The best-fit model predicts that the probability of *Plasmodium* infection of an averaged-sized *Culex* pool (17 individuals) increases from 0.10 in mid-May (~week 20) to 0.63 by late July/early August (week 31), and then declines to 0.07 by early October (week 40).

FIGURE 1. Avian malaria infection dynamics in *Culex* vectors. (A) Proportion of infected *Culex* mosquitoes across isolated putative species of Haemosporida. “Rare” infections denote CHI05PL and CHI09PL, which were each isolated only twice. “Unknown” refers to an infection that did not produce cytochrome *b* amplicon, and thus could not be confirmed as a real infection. Estimated minimum infection rates for (B) *Culex* infection rates of *Plasmodium* species specialized on American robins and (C) *Culex* infection rates of generalist *Plasmodium* lineages.

The proportion of infections in mosquito hosts assigned to putative avian *Plasmodium* species varied seasonally. Putative *Plasmodium* species CHI02PL, CHI04PL, and CHI07PL are apparent American robin specialists.^{43,44} The predicted probability of CHI02PL infection increased from near zero in mid-May (week 20) to a peak of 0.08 by late July (week 30) before declining to near zero by early October (week 40). CHI04PL was the most common parasite among mosquito pools, despite being uncommon among local robins⁴³ (prevalence = 0.06). The predicted probability of CHI04PL infection among *Culex* vector pools increased from near zero in mid-May (week 20) to 0.23 in late July (week 32), declining to near zero by early October (week 40). CHI07PL peaked slightly earlier than other robin specialists. The predicted probability of CHI07PL infection among mosquito pools increased from 0.01 in mid-May (week 20) to 0.11 in mid-July (week 28), declining to 0.01 by early October (week 40). MIRs (infected *Culex* vectors per 1,000 individuals) for CHI02PL, CHI04PL, and CHI07PL peaked at 4.1, 14.8, and 6.4, with seasonally averaged means of 1.5, 4.2, and 4.0, respectively (Figure 1B).

CHI03PL and CHI06PL, two generalized putative *Plasmodium* species with similar host ranges,^{43,44} had different

seasonal patterns of infection in *Culex* hosts. The predicted probability of CHI03PL infection among *Culex* pools increased from 0.02 in mid-May (week 20) to 0.14 in early July (week 27), declining to 0.003 by early October (week 40). In contrast, CHI06PL infections occurred later in the transmission season. The predicted probability of CHI06PL infection among mosquito pools increased from near zero in early May (week 20) to 0.08 in mid-late August (week 34), declining to 0.001 by late October (week 40). MIRs for CHI03PL and CHI06PL peaked at 8.6 and 4.7, with seasonally averaged means of 4.2 and 1.5, respectively (Figure 1C).

Haemosporidia infections in avian host populations. Seasonal *Plasmodium* infection dynamics in avian hosts differed between putative parasite species and bird species. Putative *Plasmodium* species recovered primarily from 436 American robin samples demonstrated large variation in prevalence across the transmission season, and these patterns differed between juveniles and adults (Figure 2). The best-fit multinomial logistic regression model explaining infection status in American robins included a year effect, week effect, host age effect, and an interaction effect between week and host age (weight = 1.0; Supplemental Table 6). The model revealed that across all parasite species that infect robins locally,

FIGURE 2. Avian malaria infection dynamics in a major host species. The *Plasmodium* infection status of (A) adult and (B) juvenile American robins, and the predicted probabilities of infection based on the best-fit model for (C) adult and (D) juvenile robins across the transmission season. In (C), we do not demonstrate predictions for weeks 35–40 because no adult robins were caught during that period. NI = individuals that were not infected.

overall *Plasmodium* prevalence did not vary greatly across the transmission season in adult robins (Figure 2A). However, the dynamics of individual parasite species in the robin host population were complex. Both adult and juvenile robins accumulated CHI02PL and CHI04PL over the transmission season (Figure 2). CHI02PL prevalence was greater in adults compared with juveniles (as shown in a previous study⁴⁴) and during 2007 compared with 2006. CHI04PL revealed similar dynamics across age classes, but prevalence did not vary greatly among years. Adults entered the transmission season with a high CHI07PL prevalence (May/early June prevalence = 0.58), but gradually lost infections in the circulating blood over the transmission season (Figure 2A and C). Hatch-year robins accumulated infections rapidly over time, especially between mid-June (week 25) and late August (week 35) (Figure 2B and D), and achieved comparable prevalence to adults by the end of the transmission season.

Haemosporida infection dynamics among 124 northern cardinal samples and 522 house sparrow samples did not vary substantially across the transmission season. The best-fit model explaining the infection status of northern cardinals included year and age (weight = 0.72; Supplemental Table 6), although the fit was similar to that of a model that only included age (weight = 0.27, ΔAICc = 1.94; Supplemental Table 6). Generally, Haemosporida prevalence was greater in 2006 than 2007 (Table 1). The two generalized *Plasmodium* species, CHI03PL and CHI06PL, were more prevalent among hatch-year than adult northern cardinals, while the *Parahaemoproteus* species CHI18PA was more abundant in adult northern cardinals (Table 1). The best-fit multinomial logistic regression model explaining infection status in house sparrows only included a year effect (weight = 0.80), although it was similar in fit to a model that included year and week (weight = 0.14, ΔAICc = 3.4; Supplemental Table 6). In general, *Plasmodium* infections among house sparrows were more prevalent in 2006 than 2007 (Table 1).

Mosquito infection with West Nile virus. The probability of WNV infection among 2,971 *Culex* vector pools varied seasonally and between years. The best-fit logistic regression model predicting the probability of WNV infection incorporated a quadratic effect of pool size, year and a quadratic effect of week (weight = 0.41), but was similar in fit to a model that included the same variables and an interaction between year and the quadratic week effect (ΔAICc = 0.5, AICc weight = 0.32; Supplemental Table 7). The best-fit model predicted that WNV infection probabilities were near zero until late July (week 25–26) and peaked in early August

(week 31–32). The probability of infection in *Culex* pools was approximately 1.8 times greater in 2006 than in 2007. Peak MIRs for WNV ranged between 0 and 23.2 for 2006 and 0 and 16.4 for 2007, with a seasonal average of 6.9 and 4.5, respectively (Figure 3A).

Avian WNV seroprevalence. Seasonal variation in WNV seroprevalence was similar in American robins and house sparrows. Among an identical set of candidate models, the best-fit logistic regression model for each species included effects for year, age, week, and an interaction between week and age (Supplemental Table 8). For northern cardinals, the best-fit model included a year, age, and week effect (AICc weight = 0.43), but a model that included those effects and an interaction between week and age had a similar fit to the data (weight = 0.33, ΔAICc = 0.5; Supplemental Table 8). The best-fit mixed effects logistic regression model that included all three individually analyzed host species and

FIGURE 3. West Nile virus (WNV) transmission dynamics across hosts and *Culex* vectors. (A) Estimated WNV minimum infection rate in *Culex* mosquitoes across weeks and (B) predicted WNV seroprevalence in avian hosts from a best-fit model for all common host species (random effect = host species). AHY = after-hatch-year host; HY = hatch-year host.

TABLE 1

Prevalence of CHI03PL, CHI06PL, and CHI18PA in northern cardinals house sparrows across years

	Prevalence	
	2006	2007
Northern cardinal		
CHI03PL	0.21/0.42	0.03/0.27
CHI06PL	0.13/0.38	0.09/0.27
CHI18PA	0.23/0.13	0.11/0.12
House sparrow		
CHI03PL	0.12	0.08
CHI05PL	0.04	0.03
CHI06PL	0.05	0.01

For northern cardinals, prevalence estimates in each cell are separated by age class (after-hatch-year/hatch-year host).

house finches included year, age, week effect, and an interaction between week and age (weight = 0.99; host species modeled as a random effect). Model predictions from the community-level analysis revealed that, in general, seroprevalence increased across the transmission season for both adult and juvenile hosts; however, the increase was more rapid but delayed in juveniles (Figure 3B). Among juveniles, the sharp increase in WNV seroprevalence began in late June (~week 25–26) and accelerated through July and August suggesting active transmission during this period.

Coinfection with Haemosporida and WNV in *Culex* pools. The odds of a WNV infection increased by 5.3-fold for *Culex* pools with a simultaneous *Plasmodium* infection relative to pools that lacked a *Plasmodium* infection, even after controlling for pool size as a covariate ($P < 0.01$; logistic mixed regression model, random factor = month of collection, p based on a parametric bootstrap of log-likelihood ratio). Given that these *Culex* pools included up to 36 individuals that were aggregated by site and collection date, this suggests that transmission of both avian pathogens is spatio-temporally correlated. A set of cross-correlation functions suggested that the increase in MIRs of CHI02PL, CHI03PL, and CHI07PL preceded an increase in WNV MIR (averaged across years) by 0–3, 2–5, and 0–3 weeks, respectively (Table 2). The increase in CHI06PL MIR lagged behind WNV MIR by 1–4 weeks. The transmission dynamics of CHI04PL and WNV MIR were fairly synchronous.

DISCUSSION

Seasonal infection patterns are common in vector-borne disease systems. Here, our analyses identified strong seasonal patterns in Haemosporida and WNV infection among mosquito populations and avian host communities. Interestingly, infection dynamics of various Haemosporida in *Culex* differed across the transmission season. Although the infection dynamics of CHI02PL, CHI04PL, and CHI07PL were similar to each other, CHI03PL and CHI06PL were transmitted early and late, respectively, relative to the other *Plasmodium* taxa.

The seasonal host-shift in *Culex* vectors from American robins to other common suburban birds (northern cardinals, house sparrows, and mourning doves [*Zenaida macroura*]) over the transmission season in this region⁷ might be associated with variation in the seasonal patterns of *Plasmodium* infection in mosquitoes. Mosquito feeding patterns modulate encounter rates between hosts and parasites. Nonrandom mosquito feeding patterns across host species or individuals introduces heterogeneity in the host–parasite contact rates, and has important implications for disease transmission.⁸ Indeed, parasites with similar infection dynamics in *Culex* vectors (CHI02PL, CHI04PL, and CHI07PL) were apparently spe-

cialized American robins^{43,44} and had MIRs that generally peaked in late July or early August. In addition, juvenile robins accumulated infections of these parasites contemporaneously, with the most rapid increase in infections occurring in July and August (weeks 25–35).

Large differences in the infection patterns of the two generalized putative *Plasmodium* species (CHI03PL and CHI06PL) suggest vector blood-feeding alone cannot explain the infection patterns of avian *Plasmodium* parasites in mosquito hosts. CHI03PL and CHI06PL have similar host distributions in suburban Chicago. Both parasites were prevalent in house sparrows and northern cardinals, but infrequent in American robins.⁴³ If blood-feeding patterns alone governed the transmission of these parasites to mosquito hosts, both would likely be transmitted synchronously. However, while the *Culex* MIR of CHI06PL peaked later in the transmission season, CHI03PL had the earliest increase in infection of the common *Plasmodium* parasites in the study site, especially among juvenile northern cardinals and house sparrows. For instance, over half of the juvenile northern cardinals (11/21) caught between May and mid-July were infected with CHI03PL. In addition, all nine infections of CHI03PL in American robins occurred in juveniles caught between May and mid-July. In contrast, fewer than 10% of juvenile robins (18/188) caught during this period were infected with the common robin-specialist CHI07PL.

The discordance between *Culex* feeding patterns and seasonal *Plasmodium* infection dynamics highlights the potential for other factors to drive infection patterns. Practical constraints prevented the use of molecular methods in this study to distinguish *Cx. restuans* from *Cx. pipiens* (the most likely vectors based on previous analysis⁴³). Aggregation of these morphologically similar species into pools for testing thus precluded identification of possible species differences that are also seasonally constrained (*Cx. restuans* oviposition activity typically occurs earlier than *Cx. pipiens*,^{52–54} although the two species may overlap extensively in some areas⁵⁵). Differences in the mosquito infection dynamics between CHI03PL and CHI06PL could be associated with seasonal changes in *Culex* composition if these *Culex* mosquitoes differ in their vector competence for these two *Plasmodium* species. Controlled experimental infection studies might be needed in addition to screening natural populations to describe vector competence for *Plasmodium* parasites.⁵⁶ Such studies could examine whether species of mosquito vectors that are known to share *Plasmodium* infections in nature⁵⁷ are equally effective at transmitting these parasites to hosts, and illuminate whether vector community structure can influence seasonal *Plasmodium* infection dynamics.^{58,59}

Seasonal variation in temperature may also influence patterns of *Plasmodium* transmission. The development rate of

TABLE 2

Autocorrelations of MIR time series data for each *Plasmodium* parasite and WNV with different lag values of *Plasmodium* MIR between –5 and 5 weeks

	–5 weeks	–4 weeks	–3 weeks	–2 weeks	–1 week	No lag	1 week	2 weeks	3 weeks	4 weeks	5 weeks
CHI02PL	0.16	0.49	0.75	0.91	0.93	0.80	0.51	0.17	–0.16	–0.43	–0.61
CHI03PL	0.54	0.61	0.61	0.54	0.42	0.28	0.01	–0.21	–0.43	–0.60	–0.72
CHI04PL	–0.29	0	0.33	0.64	0.88	0.99	0.89	0.67	0.36	0.03	–0.27
CHI06PL	–0.54	–0.36	–0.11	0.17	0.48	0.75	0.87	0.87	0.75	0.53	0.26
CHI07PL	0.28	0.46	0.59	0.63	0.64	0.63	0.42	0.22	–0.02	–0.25	–0.46

MIR = minimum infection rate. Cells with shade represent positive correlation strength.

some *Plasmodium* parasites in mosquitoes is strongly linked to ambient temperature.⁶⁰ In addition, various components of the mosquito immune system are temperature dependent in discordant ways.⁶¹ Both of these processes may work to influence reaction norms in vector competence across a temperature gradient. Although these reaction norms may vary between mosquito species and mosquito–parasite combinations, little is known about the influence of environmental gradients, including temperature, on vector competence across the diversity of avian *Plasmodium* parasites and mosquito vectors. Future studies on the competence of potential avian *Plasmodium* vectors should integrate environmental gradients like temperature into study designs.

The infection dynamics of CHI07PL among American robins provided general support for the classic model of temperate avian malaria transmission.³⁰ Adult American robins had a high prevalence of CHI07PL at the beginning of our sampling season in mid-May (approximately week 20). This may have been associated with prior persistent avian *Plasmodium* infections in host tissues, the recrudescence of infections into the bloodstream associated with stress from reproduction,^{15,26} and increased vector activity.⁶² Soon thereafter, the increased vector blood meals from American robins in late June⁷ may have driven the increase in the infection rate of CHI07PL among *Culex* vectors. Increasing mosquito abundance may have facilitated the transmission of these parasites to naive juvenile robins that were numerous following peak breeding, leading to an observed rapid increase in prevalence. Cumulatively, the temporal pattern of infection by CHI07PL is consistent with an age-structured bimodal peak in prevalence, in which dormant infections persist in adults through the nonbreeding season when hosts may migrate and vectors are inactive, and are subsequently transmitted to naive juveniles when infected adults return to breeding areas and vector activity resumes. Interestingly, however, not all *Plasmodium* parasites show similar dynamics,²⁹ including other robin specialist and generalist parasites in this study suggesting this transmission model may not broadly apply across the diversity of avian malaria species in temperate climates.

Our study suggests that WNV and avian *Plasmodium* have similar seasonal infection patterns. This pattern parallels that seen with *Culex* flavivirus, a mosquito specific virus that co-circulates with WNV at this same site and shows correlated patterns of transmission.⁶³ The *Culex* mosquito MIRs of both *Plasmodium* and WNV broadly overlap during the transmission season. Seasonal patterns of *Plasmodium* prevalence and WNV seroprevalence in juvenile American robins suggest that these naive hosts may accumulate infections of both pathogens contemporaneously. However, given the difficulty in interpreting the timing of infection from host serological data, we cannot preclude the possibility that birds were exposed elsewhere and subsequently immigrated to the study site. Actual coinfections are difficult to confirm with field data given the short viremic period associated with WNV infection.^{36,64} However, in a previous study³⁶ we showed that seven of 23 hosts of the species included in this analysis that had an active WNV infection were also simultaneously infected with a *Plasmodium* parasite, demonstrating that coinfections occur in this population. We also found that *Culex* vector pools infected with a *Plasmodium* parasite had a higher probability of a WNV infection. Because individuals

aggregated into these pools were captured at the same site at the same time, this association implies that areas undergoing active WNV transmission also experience active *Plasmodium* transmission. This builds on our previous work in this region that identified two individual WNV positive blood fed *Cx. pipiens* that were simultaneously infected with Haemosporida.³⁷ Cross-correlational analyses revealed that different *Plasmodium* species might have different probabilities of coinfection with WNV in vectors and host.

Our analysis suggests that various avian *Plasmodium* species and WNV co-circulate in suburban Chicago. While our study does not document interactions between *Plasmodium* parasites and WNV, it suggests that these pathogens appear to respond to similar environmental drivers. Synchronous seasonal infection patterns between *Plasmodium* and WNV promote the opportunity for direct interactions within hosts and vectors, or indirect interactions mediated by avian and insect immune systems. Previous studies have indicated that pathogen–pathogen interactions can have important impacts on disease transmission.²⁰ Indeed, ubiquitous avian *Plasmodium* infections may impact WNV transmission by influencing heterogeneity in host–vector interactions,^{65,66} the viremia profiles and survival of coinfecting hosts,³⁶ and the vectorial capacity of mosquitoes.²⁵ Furthermore, *Plasmodium* species have been shown to influence vector's biting behavior,^{66–69} and this might impact circulation of arboviruses that have similar transmission cycles. Future studies with controlled experimental designs may illuminate whether avian Haemosporida transmission can have indirect implications for public health by modulating the transmission of zoonotic pathogens.

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REFERENCES

- Altizer S, Dobson A, Hosseini P, Hudson P, Pascual M, Rohani P, 2006. Seasonality and the dynamics of infectious diseases. *Ecol Lett* 9: 467–484.
- Focks DA, Daniels E, Haile DG, Keesling JE, 1995. A simulation model of the epidemiology of urban dengue fever: literature analysis, model development, preliminary validation,

- and samples of simulation results. *Am J Trop Med Hyg* 53: 489–506.
3. Ruiz MO, Chaves LF, Hamer GL, Sun T, Brown WM, Walker ED, Haramis L, Goldberg TL, Kitron UD, 2010. Local impact of temperature and precipitation on West Nile virus infection in *Culex* species mosquitoes in northeast Illinois, USA. *Parasit Vectors* 3: 19.
 4. Tempelis CH, Washino RK, 1967. Host-feeding patterns of *Culex tarsalis* in the Sacramento Valley, California, with notes on other species. *J Med Entomol* 4: 315–318.
 5. Tempelis CH, Reeves WC, Bellamy RE, Lofy MF, 1965. A 3-year study of feeding habits of *Culex tarsalis* in Kern County, California. *Am J Trop Med Hyg* 14: 170.
 6. Tempelis CH, 1975. Host-feeding patterns of mosquitos, with a review of advances in analysis of blood meals by serology. *J Med Entomol* 11: 635–653.
 7. Hamer GL, Kitron UD, Goldberg TL, Brawn JD, Loss SR, Ruiz MO, Hayes DB, Walker ED, 2009. Host selection by *Culex pipiens* mosquitoes and West Nile virus amplification. *Am J Trop Med Hyg* 80: 268–278.
 8. Kilpatrick AM, Daszak P, Jones MJ, Marra PP, Kramer LD, 2006. Host heterogeneity dominates West Nile virus transmission. *Proc Biol Sci* 273: 2327–2333.
 9. Tempelis CH, Franczy DB, Hayes RO, Lofy MF, 1967. Variations in feeding patterns of seven culine mosquitoes on vertebrate hosts in Weld and Larimer Counties, Colorado. *Am J Trop Med Hyg* 16: 111.
 10. Burkett-Cadena ND, Hassan HK, Eubanks MD, Cupp EW, Unnasch TR, 2012. Winter severity predicts the timing of host shifts in the mosquito *Culex erraticus*. *Biol Lett* 8: 567–569.
 11. Kilpatrick AM, Kramer LD, Jones MJ, Marra PP, Daszak P, 2006. West Nile virus epidemics in North America are driven by shifts in mosquito feeding behavior. *PLoS Biol* 4: 606–610.
 12. Kim KS, Tsuda Y, 2010. Seasonal changes in the feeding pattern of *Culex pipiens pallens* govern the transmission dynamics of multiple lineages of avian malaria parasites in Japanese wild bird community. *Mol Ecol* 19: 5545–5554.
 13. Reisen WK, Cayan D, Tyree M, Barker CA, Eldridge B, Dettinger M, 2008. Impact of climate variation on mosquito abundance in California. *J Vector Ecol* 33: 89–98.
 14. Eisen L, Bolling BG, Blair CD, Beaty BJ, Moore CG, 2008. Mosquito species richness, composition, and abundance along habitat-climate-elevation gradients in the northern Colorado front range. *J Med Entomol* 45: 800–811.
 15. Allander K, 1997. Reproductive investment and parasite susceptibility in the great tit. *Funct Ecol* 11: 358–364.
 16. Nordling D, Andersson M, Zohari S, Gustafsson L, 1998. Reproductive effort reduces specific immune response and parasite resistance. *Proc Biol Sci* 265: 1291–1298.
 17. Hamer GL, Walker ED, Brawn JD, Loss SR, Ruiz MO, Goldberg TL, Schotthoefer AM, Brown WM, Wheeler E, Kitron UD, 2008. Rapid amplification of West Nile virus: the role of hatch-year birds. *Vector Borne Zoonotic Dis* 8: 57–67.
 18. Janousek WM, Marra PP, Kilpatrick AM, 2014. Avian roosting behavior influences vector-host interactions for West Nile virus hosts. *Parasit Vectors* 7: 399.
 19. Krebs BL, Anderson TK, Goldberg TL, Hamer GL, Kitron UD, Newman CM, Ruiz MO, Walker ED, Brawn JD, 2014. Host group formation decreases exposure to vector-borne disease: a field experiment in a ‘hotspot’ of West Nile virus transmission. *Proc Biol Sci* 281: 20141586.
 20. Cox FEG, 2001. Concomitant infections, parasites and immune responses. *Parasitology* 122: S23–S38.
 21. Kenney JL, Brault AC, 2014. The role of environmental, virological and vector interactions in dictating biological transmission of arthropod-borne viruses by mosquitoes. *Adv Virus Res* 89: 39–83.
 22. Ezenwa VO, Etienne RS, Luikart G, Beja-Pereira A, Jolles AE, 2010. Hidden consequences of living in a wormy world: nematode-induced immune suppression facilitates tuberculosis invasion in African Buffalo. *Am Nat* 176: 613–624.
 23. Johnson PTJ, Hoverman JT, 2012. Parasite diversity and coinfection determine pathogen infection success and host fitness. *Proc Natl Acad Sci USA* 109: 9006–9011.
 24. Vaughan JA, Turell MJ, 1996. Dual host infections: enhanced infectivity of eastern equine encephalitis virus to *Aedes* mosquitoes mediated by *Brugia microfilariae*. *Am J Trop Med Hyg* 54: 105–109.
 25. Vaughan JA, Turell MJ, 1996. Facilitation of Rift Valley fever virus transmission by *Plasmodium berghei* sporozoites in *Anopheles stephensi* mosquitoes. *Am J Trop Med Hyg* 55: 407–409.
 26. Valkiunas G, 2005. *Avian Malaria Parasites and Other Haemosporidia*. Boca Raton, FL: CDC Press.
 27. Ellis VA, Cornet S, Merrill L, Kunkel MR, Tsunekage T, Ricklefs RE, 2015. Host immune responses to experimental infection of *Plasmodium relictum* (lineage SGS1) in domestic canaries (*Serinus canaria*). *Parasitol Res* 114: 3627–3636.
 28. Applegate JE, Beaudoin RL, 1970. Mechanism of spring relapse in avian malaria: effect of gonadotropin and corticosterone. *J Wildl Dis* 6: 443–447.
 29. Cosgrove CL, Wood MJ, Day KP, Sheldon BC, 2008. Seasonal variation in *Plasmodium* prevalence in a population of blue tits *Cyanistes caeruleus*. *J Anim Ecol* 77: 540–548.
 30. Beaudoin RL, Applegate JE, Davis DE, McLean RG, 1971. A model for the ecology of avian malaria. *J Wildl Dis* 7: 5–13.
 31. Lanciotti RS, Roehrig JT, Deubel V, Smith J, Parker M, Steele K, Crise B, Volpe KE, Crabtree MB, Scherret JH, Hall RA, MacKenzie JS, Cropp CB, Panigrahy B, Ostlund E, Schmitt B, Malkinson M, Banet C, Weissman J, Komar N, Savage HM, Stone W, McNamara T, Gubler DJ, 1999. Origin of the West Nile virus responsible for an outbreak of encephalitis in the northeastern United States. *Science* 286: 2333–2337.
 32. Murray KO, Mertens E, Despres P, 2010. West Nile virus and its emergence in the United States of America. *Vet Res* 41: 67.
 33. LaDeau SL, Kilpatrick AM, Marra PP, 2007. West Nile virus emergence and large-scale declines of North American bird populations. *Nature* 447: 710–713.
 34. Wimberly MC, Giacomo P, Kightlinger L, Hildreth MB, 2013. Spatio-temporal epidemiology of human West Nile virus disease in South Dakota. *Int J Environ Res Public Health* 10: 5584–5602.
 35. Hahn MB, Monaghan AJ, Hayden MH, Eisen RJ, Delorey MJ, Lindsey NP, Nasci RS, Fischer M, 2015. Meteorological conditions associated with increased incidence of West Nile virus disease in the United States, 2004–2012. *Am J Trop Med Hyg* 92: 1013–1022.
 36. Medeiros MCI, Anderson TK, Higashiguchi JM, Kitron UD, Walker ED, Brawn JD, Krebs BL, Ruiz MO, Goldberg TL, Ricklefs RE, Hamer GL, 2014. An inverse association between West Nile virus serostatus and avian malaria infection status. *Parasit Vectors* 7: 415.
 37. Boothe E, Medeiros MCI, Kitron UD, Brawn JD, Ruiz MO, Goldberg TL, Walker ED, Hamer GL, 2015. Identification of avian and hemoparasite DNA in blood-engorged abdomens of *Culex pipiens* (Diptera: Culicidae) from a West Nile virus epidemic region in suburban Chicago, Illinois. *J Med Entomol* 52: 461–468.
 38. Harrington LC, Poulson RL, 2008. Considerations for accurate identification of adult *Culex restuans* (Diptera: Culicidae) in field studies. *J Med Entomol* 45: 1–8.
 39. Fallon SM, Ricklefs RE, Swanson BL, Bermingham E, 2003. Detecting avian malaria: an improved polymerase chain reaction diagnostic. *J Parasitol* 89: 1044–1047.
 40. Fecchio A, Lima MR, Svensson-Coelho M, Marini MA, Ricklefs RE, 2013. Structure and organization of an avian haemosporidian assemblage in a neotropical savanna in Brazil. *Parasitology* 140: 181–192.
 41. Outlaw DC, Ricklefs RE, 2014. Species limits in avian malaria parasites (Haemosporida): how to move forward in the molecular era. *Parasitology* 141: 1223–1232.
 42. Svensson-Coelho M, Blake JG, Loisele BA, Penrose AS, Parker PG, Ricklefs RE, 2013. Diversity, prevalence, and host specificity of avian *Plasmodium* and *Haemoproteus* in a western Amazon assemblage. *Ornithol Monogr* 76: 1–47.
 43. Medeiros MCI, Hamer GL, Ricklefs RE, 2013. Host compatibility rather than vector-host-encounter rate determines the host range of avian *Plasmodium* parasites. *Proc Biol Sci* 280: 20122947.

44. Medeiros MC, Ellis VA, Ricklefs RE, 2014. Specialized avian Haemosporida trade reduced host breadth for increased prevalence. *J Evol Biol* 27: 2520–2528.
45. Mckee EM, Walker ED, Anderson TK, Kitron UD, Brawn JD, Krebs BL, Newman C, Ruiz MO, Levine RS, Carrington ME, McLean RG, Goldberg TL, Hamer GL, 2015. West Nile virus antibody decay rate in free-ranging birds. *J Wildl Dis* 51: 601–608.
46. Chaves LF, Hamer GL, Walker ED, Brown WM, Ruiz MO, Kitron UD, 2011. Climatic variability and landscape heterogeneity impact urban mosquito diversity and vector abundance and infection. *Ecosphere* 2: 1–21.
47. Loss SR, Hamer GL, Walker ED, Ruiz MO, Goldberg TL, Kitron UD, Brawn JD, 2009. Avian host community structure and prevalence of West Nile virus in Chicago, Illinois. *Oecologia* 159: 415–424.
48. Lanciotti RS, Kerst AJ, Nasci RS, Godsey MS, Mitchell CJ, Savage HM, Komar N, Panella NA, Allen BC, Volpe KE, Davis BS, Roehrig JT, 2000. Rapid detection of West Nile virus from human clinical specimens, field-collected mosquitoes, and avian samples by a TaqMan reverse transcriptase-PCR assay. *J Clin Microbiol* 38: 4066–4071.
49. Wickham H, 2009. *ggplot2: Elegant Graphics for Data Analysis*. New York, NY: Springer-Verlag.
50. Ishtiaq F, Guillaumot L, Clegg SM, Phillimore AB, Black RA, Owens IPF, Mundy NI, Sheldon BC, 2008. Avian haematozoan parasites and their associations with mosquitoes across south-west Pacific Islands. *Mol Ecol* 17: 4545–4555.
51. Njabo KY, Cornel AJ, Bonneaud C, Toffelmier E, Sehgal RNM, Valkiunas G, Russell AF, Smith TB, 2011. Nonspecific patterns of vector, host and avian malaria parasite associations in a central African rainforest. *Mol Ecol* 20: 1049–1061.
52. Lee JH, Rowley WA, 2000. The abundance and seasonal distribution of *Culex* mosquitoes in Iowa during 1995–97. *J Am Mosq Control Assoc* 16: 275–278.
53. Andreadis TG, Anderson JF, Vossbrinck CR, 2001. Mosquito surveillance for West Nile virus in Connecticut, 2000: isolation from *Culex pipiens*, *Cx. restuans*, *Cx. salinarius*, *Culiseta melanura*. *Emerg Infect Dis* 7: 670–674.
54. Jackson BT, Paulson SL, Youngman RR, Scheffel SL, Hawkins B, 2005. Oviposition preferences of *Culex restuans* and *Culex pipiens* (Diptera: Culicidae) for selected infusions in oviposition traps and gravid traps. *J Am Mosq Control Assoc* 21: 360–365.
55. Jackson BT, Paulson SL, 2006. Seasonal abundance of *Culex restuans* and *Culex pipiens* in southwestern Virginia through ovitrapping. *J Am Mosq Control Assoc* 22: 206–212.
56. Žiegytė R, Valkiūnas G, 2015. Recent advances in vector studies of avian haemosporidian parasites. *Ekologija* 60: 73–83.
57. Kimura M, Darbro JM, Harrington LC, 2010. Avian malaria parasites share congeneric mosquito vectors. *J Parasitol* 96: 144–151.
58. Kim KS, Tsuda Y, 2012. Avian *Plasmodium* lineages found in spot surveys of mosquitoes from 2007 to 2010 at Sakata wetland, Japan: do dominant lineages persist for multiple years? *Mol Ecol* 21: 5374–5385.
59. Carlson JS, Walther E, TroutFryxell R, Staley S, Tell LA, Sehgal RNM, Barker CM, Cornel AJ, 2015. Identifying avian malaria vectors: sampling methods influence outcomes. *Parasit Vectors* 8: 365.
60. LaPointe DA, Goff ML, Atkinson CT, 2010. Thermal constraints to the sporogonic development and altitudinal distribution of avian malaria *Plasmodium relictum* in Hawaii. *J Parasitol* 96: 318–324.
61. Murdock CC, Paaijmans KP, Bell AS, King JG, Hillyer JF, Read AF, Thomas MB, 2012. Complex effects of temperature on mosquito immune function. *Proc Biol Sci* 279: 3357–3366.
62. Cornet S, Nicot A, Rivero A, Gandon S, 2014. Evolution of plastic transmission strategies in avian malaria. *PLoS Pathog* 10: e1004308.
63. Newman CM, Cerutti F, Anderson TK, Hamer GL, Walker ED, Kitron UD, Ruiz MO, Brawn JD, Goldberg TL, 2011. *Culex* flavivirus and West Nile virus mosquito coinfection and positive ecological association in Chicago, United States. *Vector Borne Zoonotic Dis* 11: 1099–1105.
64. Komar N, Langevin S, Hinten S, Nemeth N, Edwards E, Hettler D, Davis B, Bowen R, Bunning M, 2003. Experimental infection of North American birds with the New York 1999 strain of West Nile virus. *Emerg Infect Dis* 9: 311–322.
65. Cornet S, Nicot A, Rivero A, Gandon S, 2013. Both infected and uninfected mosquitoes are attracted toward malaria infected birds. *Malar J* 12: 179.
66. Cornet S, Nicot A, Rivero A, Gandon S, 2013. Malaria infection increases bird attractiveness to uninfected mosquitoes. *Ecol Lett* 16: 323–329.
67. Lalubin F, Bize P, van Rooyen J, Christe P, Glaizot O, 2012. Potential evidence of parasite avoidance in an avian malarial vector. *Anim Behav* 84: 539–545.
68. Koella JC, Sorensen FL, Anderson RA, 1998. The malaria parasite, *Plasmodium falciparum*, increases the frequency of multiple feeding of its mosquito vector, *Anopheles gambiae*. *Proc Biol Sci* 265: 763–768.
69. Lacroix R, Mukabana WR, Gouagna LC, Koella JC, 2005. Malaria infection increases attractiveness of humans to mosquitoes. *PLoS Biol* 3: 1590–1593.