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Elucidating the Factors that Modulate the Distribution of Avian Haemosporida Parasites across a Community of Hosts

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Dissertation Summary

Parasites are heterogeneously distributed across host species, host populations, and host individuals within populations. A primary aim of infectious disease ecology seeks to uncover the factors that drive this heterogeneity. At a fundamental level, host infection is determined by exposure and susceptibility to a pathogen. My dissertation explores how evolutionary and ecological forces associated with these fundamental determinates of infection shape variation in parasite host breadth and host infection status. Here, I focus on a community of vector-borne avian Haemosporida parasites among suburban birds of Chicago, IL. These parasites exhibit strong variation in their distribution among available hosts, and are an ideal system to investigate factors that structure parasite-host interactions.

Numerous studies have shown that Haemosporidia-bird interactions are highly idiosyncratic, with specific parasite taxa infecting only a subset of available hosts. However, it remained unclear if this was the result of differential compatibilities between alternate combinations of host-parasite pairs, or whether heterogeneous feeding patterns of vectors structured parasite-host relationships by limiting the access of parasites to discrete subsets of hosts. In chapter one, I show that although *Plasmodium* parasites are heterogeneously distributed across their avian hosts, the feeding patterns of the two dominant vectors were similar and did not explain variation in *Plasmodium* host range. However, phylogenetic similarity between host species predicted similarity in their *Plasmodium* parasites. Cumulatively, this suggests that host compatibility issues that exist solely between the host and parasite structure avian *Plasmodium* host range.

Heterogeneity in Haemosporida parasite-bird interactions is often revealed by the large variation in the prevalence that exists among host species within a community. Biologists have used this variation in total avian Haemosporida prevalence among host species to test broad hypotheses that link ecological attributes to disease risk. However, with little information on vector-host interactions, such studies did not address the encounter rate between host and parasites directly as a causative factor driving variation in avian Haemosporida prevalence. In chapter 2, I show that the feeding patterns of the two most dominant *Plasmodium* vectors in suburban Chicago, IL are heterogeneous across available bird species. Moreover, host species that are over-utilized by vectors have a higher prevalence of *Plasmodium.* However, this effect was not consistently present when individual *Plasmodium* taxa were analyzed separately. I argue that vector utilization rates may set an upper limit to *Plasmodium* prevalence, under which processes associated with host parasite coevolution and parasite competition may modulate more restricted host ranges of individual taxa. In addition, the results caution against relating total *Plasmodium* prevalence to species traits without controlling for direct exposure to vectors.

Interactions with other pathogens may influence the distribution of a parasite across hosts through direct competition for resources or indirectly as mediated by host physiology. In chapter 3, I investigate whether previous infection with West Nile virus, a common pathogen of surburban North American birds, influences the probability of Haemosporida infection. I demonstrate a negative association between West Nile virus serostatus and *Plasmodium* infection status. However, this relationship was found only among adult birds and was absent for *Haemoproteus*. The

mechanisms that drive this effect are difficult to determine without controlled experimental infection techniques. However, it may imply that chronic *Plasmodium* infections disappear from the blood stream as a result of direct competition with West Nile virus following infection, or concomitant infection may cause an increased probability of host mortality.

Some Haemosporida taxa are primarily restricted to a single host species, including numerous *Plasmodium* and *Haemoproteus* among the suburban passerines sampled here. The evolution of such specialization is perplexing since it involves a reduction in the available amount of hosts. However, specialists may compensate for this obvious cost by being more efficient on their hosts. In chapter 4, I explore a tradeoff between host breadth and average host efficiency across generalist and specialist Haemosporida parasites. I show that specialist parasites are more abundant than generalists among shared hosts. Moreover, the estimated density of hosts with infections did not vary significantly with host breadth, suggesting specialists and generalists may infect a similar number of host individuals. Lastly, I show that generalists primarily infect juvenile birds while specialists tend to infect adult hosts. I argue that this may reflect the ability for specialists and generalists to persist in their host following infection.

My dissertation reveals factors that contribute to the modulation of Haemosporida distributions across available hosts. While total *Plasmodium* prevalence was related to encounters with vectors, the distribution of discrete parasite taxa appears to be largely independent of a vector-imposed encounter rate. Moreover, host breadth is constrained by the phylogenetic similarity of hosts. This suggests host

compatibility and susceptibility plays a larger role in regulating host breadth than exposure. At the individual level, infections with other pathogens and age class relate to the probability of infection with different lineages of Haemosporida parasites. Cumulatively, my dissertation suggests that coevolutionary forces between parasites, and parasites and their hosts shape avian haemosporidian host ranges, perhaps under a tradeoff between host breadth and the ability to reproduce and persist within hosts. These results underscore the suitability of avian Haemosporida to investigate parasite host breadth evolution, and suggest that experimental infection studies within this system may yield important insights into the evolutionary ecology of parasite-host interactions.

Chapter 1

Host Compatibility Rather than Vector-Host Encounter Rate Determines the Host Range of Avian *Plasmodium* Parasites

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Summary

Blood-feeding arthropod vectors are responsible for transmitting many parasites between vertebrate hosts. While arthropod vectors often feed on limited subsets of potential host species, little is known about the extent to which this influences the distribution of vector-borne parasites in some systems. Here, we test the hypothesis that different vector species structure parasite-host relationships by restricting access of certain parasites to a subset of available hosts. Specifically, we investigate how the feeding patterns of *Culex* mosquito vectors relate to distributions of avian malaria parasites among hosts in suburban Chicago, IL, USA. We show that *Plasmodium* lineages, defined by cytochrome *b* haplotypes, are heterogeneously distributed across avian hosts. However, the feeding patterns of the dominant vectors (*Culex restuans* and *Culex pipiens*) are similar across these hosts, and do not explain the distributions of *Plasmodium* parasites. Phylogenetic similarity of avian hosts predicts similarity in their *Plasmodium* parasites. This effect was driven primarily by the general association of *Plasmodium* parasites with particular host superfamilies. Our results

suggest that a mosquito-imposed encounter rate does not limit the distribution of avian *Plasmodium* parasites across hosts. This implies that compatibility between parasites and their avian hosts structure *Plasmodium* host range.

keywords: avian malaria, *Plasmodium*, host encounter rate, host compatibility, mosquito-feeding patterns, host range

Introduction

Parasites are heterogeneously distributed across hosts (Poulin 2007). This heterogeneity in host distribution can arise due to (1) variability in the frequency of encounters between hosts and parasites and (2) the ability of parasites to invade and persist on the hosts they encounter (Combes 1991). Combes 1991 described these ecological drivers of host distribution as the encounter and host compatibility filters, respectively. Assessing the relative strength of these filters is a fundamental step in determining mechanisms that govern the distribution of a parasite across hosts. Understanding factors that modulate host range is important because changes in these factors alter transmission dynamics (Kilpartick *et al.* 2006a, Simpson *et al.* 2012, Allan *et al.* 2010) and introduce novel parasites to naive hosts, sometimes with devastating consequences (van Riper *et al.* 1986).

Previous studies have empirically demonstrated that both the encounter and host compatibility filter can be important obstacles for host infection. Studies commonly assess the strength of these filters by controlling for the encounter filter through experimental infection. These demonstrate that parasites differ in their

compatibility with hosts (Palinauskas *et al.* 2008, Komar *et al.* 2003, Glbert and Webb 2007), and that many are capable of infecting hosts outside their natural host range (Pearlman and Jaenike 2003). Infection probabilities on novel hosts can increase with phylogenetic relatedness with the original host, suggesting that the compatibility filter strengthens with increasing host phylogenetic distance (Glbert and Webb 2007, Pearlman and Jaenike 2003). Measuring the encounter filter directly in nature can be logistically difficult, however studies that have done so reveal interesting patterns. Strong encounter filters can mask the influence of the host compatibility filter if less susceptible host species experience more encounters with parasites (Detwiler and Minchella 2009). Strong encounter filters can exist in spite of high host-parasite sympatry. Unparasitised host species can occur in close proximity to highly parasitised host species (Kuris *et al.* 2007), suggesting fine-tuning in the mechanisms of parasites to encounter hosts, and of hosts to evade them.

Vectors control host encounters for a diversity of parasites and provide a convenient way to measure encounter rates in nature. Many arthropod vectors transmit parasites between vertebrate hosts during blood-feeding activities. Thus, blood-feeding patterns effectively set the encounter rate between vector-borne parasites and hosts. Mosquitoes, which are important vectors for a diversity of pathogens, are known to feed heterogeneously across hosts by utilising some species disproportionately relative to their abundance (Kilpatrick *et al.* 2006b, Hamer *et al.* 2009, Hassan *et al.* 2003). This heterogeneity in mosquito-feeding patterns can strongly influence disease transmission dynamics (Kilpartick *et al.* 2006a, Simpson *et al.* 2012, Kilpartick *et al.* 2006b, Hamer *et al.* 2011). Mosquito feeding networks may

also be compartmentalised (Graham *et al.* 2009), with certain vector species utilising a distinct subset of available host species (Hamer *et al.* 2009, Molaei *et al.* 2008, Malmqvist *et al.* 2004, Helgren *et al.* 2008, Kim and Tsuda 2012). For instance in the northeastern United States, *Culex restuans*, *Culex pipiens*, and *Culisetta melanura* obtain blood meals from birds, while the sympatric *Aedes vexans, Ochlerotatus,* and *Anopheles* species rely primarily on mammals for blood meals (Molaei *et al.* 2008, Molaei *et al.* 2006a, Molaei *et al.* 2006b).

Compartmentalisation in vector feeding patterns across hosts may serve as an ecological barrier to transmission, and limit access of vector-borne parasites to different suites of hosts (Helgren *et al.* 2008). In a community of hosts, vectors, and vector-borne parasites, vector species can impose a limiting encounter filter for parasites by feeding on non-overlapping or weakly overlapping subsets of potential hosts (Helgren *et al.* 2008, Gager *et al.* 2008). These subsets form compartments in an interaction network that summarises the feeding patterns of vectors on host species. If this network defines the routes parasites take to move between hosts, parasites would move more readily between hosts that exist within a compartment than between hosts that occupy different compartments. Accordingly, this would tend to homogenise parasite assemblages across host species that share the same compartments in the mosquito-host network. This model suggests an easily testable hypothesis, namely that host species fed upon by the same vector species harbour the same parasite species.

Avian malaria parasites of the genus *Plasmodium* provide a suitable system to investigate the impact of vector feeding behaviour in delimiting the host range of a parasite. Avian *Plasmodium* parasites have complex life cycles, which include asexual

stages of reproduction in a bird host and sexual stages of reproduction within a mosquito vector (Valkiūnas 2005). Briefly, the life cycle within the mosquito begins when gametocytes from an infectious bird are ingested during a blood meal. These gametocytes differentiate into gametes that fuse to form ookinetes in the mosquito midgut. Ookinetes develop into oocysts that attach to the midgut wall. Sporozoites develop within oocycts. Once released, they selectively invade the mosquito's salivary glands. Successful transmission between birds occurs when a mosquito survives long enough for the parasite to proceed through this lifecycle and injects sporozoites into another bird upon taking a subsequent blood meal.

Despite the potential importance of vectors in structuring *Plasmodium*-host relationships, most studies have focused on characterising the diversity of *Plasmodium* infections in avian hosts (Bensch *et al.* 2000, Fallon *et al.* 2003a, 2005, Ricklefs *et al.* 2005, Beadell *et al.* 2004, Beadell *et al.* 2009, Latta and Ricklefs 2010). The identification of the vectors in these systems has lagged behind (but see Kim and Tsuda 2012, Gager *et al.* 2008, Ishtiaq *et al.* 2008, Njabo *et al.* 2009, Njabo *et al.* 2011, Kimura *et al.* 2010). Even fewer studies have investigated the role of vectors in the transmission process and in the evolutionary biology of these parasites (but see Kim and Tsuda 2010, 2012, Gager *et al.* 2008, Svensson-Coelho and Ricklefs 2011). However, many studies hypothesise that vector dynamics may explain distributional patterns of these parasites (Kim and Tsuda 2010, 2012, Wood *et al.* 2007, Kimura *et al.* 2006).

Patterns of avian *Plasmodium* host range are highly idiosyncratic (Bensch *et al.* 2000, Fallon *et al.* 2003a, 2005, Svensson-Coelho and Ricklefs 2011, Ricklefs *et*

al. 2011). *Plasmodium* parasites are non-randomly distributed across host species, typically infecting only a subset of available hosts (Bensch *et al.* 2000, Fallon *et al.* 2005). Some avian *Plasmodium* taxa are nearly restricted to a single host species (Ricklefs *et al.* 2005, Latta and Ricklefs 2010). In addition, these relationships can vary geographically, and *Plasmodium* parasites may occur on different hosts across their range (Fallon *et al.* 2005, Ricklefs *et al.* 2011). These host-parasite relationships are not well preserved through time (Fallon *et al.* 2004), and co-phylogenetic analyses of parasites and hosts reveal that host switching over evolutionary time-scales is pervasive (Ricklefs and Fallon 2002, Ricklefs *et al.* 2004). These geographically variable relationships and host-switching events suggest that avian *Plasmodium* parasites have the ability to evolve the necessary machinery to exploit a broad range of hosts, despite their restricted host ranges at any given point in space and time. This raises the possibility that an encounter filter imposed by modular mosquito-feeding patterns could account for this apparent contradiction, by restricting access to only a subset of hosts that can be exploited by an avian *Plasmodium* parasite (Gager *et al.* 2008).

The topic has been approached before within the avian *Plasmodium* system. Gager *et al.* 2008 integrated information on the distribution of *Plasmodium* lineages across vectors and the avian host *Turdus grayi* in central Panama. They discovered that two common *Plasmodium* lineages of *T. grayi* occurred in different vector species, demonstrating that the two species of vectors feed on *T. grayi*. In addition, the vectors carried many *Plasmodium* lineages that were not isolated from *T. grayi* despite access to this host. The study did not support the existence of a limiting encounter

filter because *T. grayi* were exposed to both vectors and all the avian malaria lineages in the study area, but only a subset of *Plasmodium* lineages were found to infect *T. grayi* individuals. However, the study was limited to a single avian host, did not resolve the feeding patterns of vectors, and did not explore the hypothesis in a community context.

Here, we evaluate the influence of mosquito vectors in modulating the distribution of specific *Plasmodium* taxa across a community of avian hosts in suburban Chicago, IL, USA. Specifically, we identify local avian *Plasmodium* vectors and use a series of analyses to investigate whether their feeding patterns influence how *Plasmodium* parasites are distributed across avian hosts. We also investigate the potential for host compatibility to structure these relationships. Cumulatively, we assess the relative strength of a mosquito imposed encounter filter and compatibility filter in delimiting the distribution of avian *Plasmodium* parasites across a host community in an effort to understand factors that influence parasite host range. We find mosquito-feeding patterns do not explain the heterogeneous distributions of *Plasmodium* parasites across avian hosts, suggesting that host compatibility issues dominate processes that structure parasite host range in this system.

Methods

Study system and sampling

The study was conducted in 17 scattered suburban sites including parks, cemeteries, and residential communities in Chicago, IL, USA (Loss *et al.* 2009).

Avian blood samples were collected from May through September during 2006 and 2007. Mosquito samples were collected with canopy-level CDC light traps (see Chaves *et al.* 2011) from June through September during the same years at 13 of the 17 sites in which birds were captured.

Resolving Mosquito-feeding Patterns

Mosquito feeding patterns were resolved by Hamer *et al.* 2009. The study identified the vertebrate source of 1043 blood meals of 9 mosquito species in suburban Chicago. Six of the mosquito species were observed to feed on birds. However, only *Culex pipiens*, *Culex restuans* and *Aedes vexans* were well sampled, fed on birds, and were abundant within the study area (Chaves *et al.* 2011). Avian blood meals were recovered from 488 *Culex pipiens,* 172 *Culex restuans,* and 15 *Aedes vexans* individuals sampled from 2005-2007. An additional 75 *Culex pipiens* and 77 *Culex restuans* from 2008-2009 were added to the analysis presented here. Molecular procedures for identifying *Culex* blood meals may be found in Hamer *et al.* 2009. Engorged mosquitoes were sampled in the same study sites at which both avian hosts and mosquito vectors were surveyed for parasites. While *Culex pipiens* represents a well-known species complex, previous study showed that introgression of *molestus* and *quiquefasciatus* forms is minimal in the Chicago population (Huang *et al.* 2009). Thus, the numerous behavioural and physiological differences between these forms (Fonseca *et al.* 2004) are unlikely to influence the patterns presented here.

Resolving Parasite-Bird and Parasite-Mosquito Relationships

Avian hosts were sampled using standard mist netting protocols. Blood was obtained by jugular venepuncture and was stored in BA-1 dilutent or Longmire's Lysis Buffer at < -20°C. A subsample of 10 μL was used to extract DNA using an ammonium acetate protein precipitation procedure. Samples were purified through a standard isopropanol precipitation followed by two consecutive washes with 70% ethanol. Samples were eluted in double-distilled PCR-grade water for at least 3 days before further processing. DNA samples were screened for the presence of haemosporidian parasites through a polymerase chain reaction (PCR) that targeted a small segment of the 16S rRNA gene (Fallon *et al.* 2003b). Samples that screened positive with the 16S rRNA primers were used in a secondary nested PCR that targeted a 552-base pair fragment of the haemosporidian cytochrome *b* gene. Details of this reaction are presented by Fecchio *et al.* 2013. The fragment was sequenced to identify the haemosporidian responsible for the infection.

The taxonomy of avian Haemosporida is controversial and currently unresolved. Traditionally, subtle morphological characters were used to distinguish taxa (Valkiūnas 2005). However recent studies have demonstrated substantial genetic diversity within some morphospecies, and have raised the possibility of cryptic species in this system (Beadell *et al.* 2009, Bensch *et al.* 2004, Martinsen *et al.* 2006, 2007). However, the status of most haemosporidian parasites as biological species remains untested. Thus, no species level of genetic divergence can be established. In addition, reliable independent nuclear markers are not available to identify isolated lineages by linkage disequilibrium criteria (Bensch *et al.* 2004). Here, we delimit evolutionary independent parasite lineages based on the similarity of cytochrome *b* haplotypes in a

manner similar to Ricklefs *et al.* 2005. Evolutionary independent lineages are defined as the set of closely related (<1% sequence divergence) monophyletic parasite mitochondrial haplotypes recovered from the same host species or set of host species. Cytochrome b haplotypes of *Plasmodium* lineages identified in this manuscript are deposited in GenBank (accession numbers KC789821-28).

Three mosquito species (*Aedes vexans*, *Culex pipiens*, and *Culex restuans*) that were abundant (Chaves *et al.* 2011) and observed to feed on birds in Chicago (Hamer *et al.* 2009) were screened for the presence of *Plasmodium* parasites. Previous research has demonstrated that these *Culex* species are known avian malaria vectors (Valkiūnas 2005) and are infected with many of the same avian *Plasmodium* lineages (Kimura *et al.* 2010). Little information exists on the vectorial capacity of *Aedes vexans*. This species was included in this parasite survey because it fed on birds and was abundant in the study site (Chaves *et al.* 2011). Individuals were pooled by species, site, and date of capture. Pool sizes varied from 1 to 36 whole-bodied individuals. *Culex pipiens* and *Culex restuans* are not reliably distinguished based on morphology (Harrington *et al.* 2008). Due to the time and expense of the molecular diagnostics to distinguish these species (Crabtree *et al.* 1995), the *Culex* species were pooled together. DNA was extracted from mosquito pools using Qiagen blood and tissue kits following the manufacturer's protocol. Mosquito DNA samples were screened and haemosporidian infections were identified using the same molecular procedures for bird hosts. Maximum likelihood estimates of the infection rate in mosquitoes were calculated with the PoolInfRate (www.cdc.gov), version 4.0 add-in for Microsoft Excel (Biggerstaff 2012).

Because whole-bodied mosquitoes were used, we cannot distinguish the proportion of mosquitoes that had infectious sporozoites, which typically occupy the salivary glands in the thorax, from those that had ookinete or oocysts infections within the midgut (Valkiūnas 2005). We assume that the proportion of infected mosquitoes is correlated with the proportion of infectious mosquitoes across different *Plasmodium* lineages. This assumption is supported by Ishtiaq *et al.* 2008, who demonstrated that *Plasmodium* prevalence from mosquito thorax isolations was statistically indistinguishable from abdominal isolations in wild mosquitoes collected across southwest Pacific Islands.

Host Phylogenetic Distance Estimates

Phylogenetic distances between hosts were estimated with a phylogenetic tree based on a 656-bp fragment of the recombination-activating gene 1 (RAG1). A maximum likelihood gene tree was constructed using the PHYML plug-in in the program Geneious (Guindon and Gascuel 2003). The resulting topology was similar to that of Barker *et al.* 2002. See the electronic supplementary material (Supplementary Material, Section 2) for more information. Novel RAG1 sequences obtained for this study are deposited in GenBank (accession numbers KC789829-33).

Statistical Analyses

All analyses performed here focus on 10 commonly sampled avian host species with 7 or more infections of one or more of 7 commonly sampled *Plasmodium*

lineages (summarised in Table 1). Two *Plasmodium* cytochrome *b* haplotypes were identical to those of known *Plasmodium* morphospecies: *Plasmodium cathemerium* (AY377128, Wiersch *et al.* 2005) and *Plasmodium elongatum* (AY733088, Valkiūnas *et al.* 2008). These lineages are referred to by their scientific name. The mosquito-feeding patterns of the two *Culex* species across the 10 common avian *Plasmodium* hosts were compared with a G-test. One was added to each cell to avoid problems associated with zero cell values.

Mantel tests were used to assess whether 1) pairwise similarities in relationships between hosts and mosquitoes inferred from the blood-feeding patterns, and 2) phylogenetic distance between host species, were associated with pairwise similarity in the distribution of *Plasmodium* parasites across all pairwise combinations of host species. This statistical test measures the correlation between two equivalent distance matrices and assesses significance through a process of permutation. Each matrix used in the two Mantel tests placed the seven host species along rows and columns. The Morisita-Horn quantitative similarity index was used to estimate similarity in both the relationships with mosquitoes and *Plasmodium* parasites between host pairs. The Morisita-Horn quantitative similarity index was chosen because it best handled variation in the number of identified *Plasmodium* infections between hosts involved in a comparison. Morisita-Horn distances were computed using the *vegan* package in program R. Phylogenetic distance between host pairs was based on phylogenetic branch lengths (see Supplementary Material, Section2). Results did not change when per cent sequence divergence was used instead of patristic distances. For both Mantel tests, a significance test of the association between the

matrices was based on 10,000 randomised permutations. Mantel tests were performed in program R using the *vegan* package.

Similarity between parasite assemblages was visualised using non-metric multidimensional scaling. The number of dimensions was determined by the elbow test based on the relationship between the STRESS of an individual ordination and the number of dimensions. STRESS is the proportion of the residual sum of squares of the deviations from a monotonic regression of observed on predicted distances of species in ordination space. There was a dramatic reduction in STRESS (0.002 to <0.0001), between ordinations with 2 and 3 dimensions in the analysis with a marginal reduction (<0.0001) between 3 and 4 dimensions. Thus, three dimensions were used in the analysis. Pairwise similarities between parasite assemblages on host species and the mosquito vectors were compared statistically using G-tests. All G-tests were conducted in Microsoft Excel using the pop tools v 3.2.5 add-in [http://www.poptools.org].

A Monte Carlo approach was used to simulate the distributions of each *Plasmodium* lineage across host species. Three separate simulations were performed, each with a unique set of assumptions (Supplementary Material, Section S3). All simulations were run in program R, using the function "rmultinom" to generate multinomially distributed random number vectors based on a specified probability distribution. The expected value (the mean of the simulated values) and the 5% confidence limits for each *Plasmodium*-host pair were extracted from the vectors. More information is presented in the Supplementary Material, Section 3.

Results

All 7 common *Plasmodium* lineages recovered from avian hosts were discovered in *Culex* mosquito pools. Maximum likelihood estimates of mosquito infection rates for each *Plasmodium* lineage are presented in Table 1. *Plasmodium* parasites were not detected among *Aedes vexans* pools. The mosquito feeding patterns and the parasite screening results suggest *Culex pipiens* and *Culex restuans* are the major *Plasmodium* vectors in Chicago. Thus*, Aedes vexans* was not included in subsequent analyses.

Patterns of avian host use did not differ significantly between of *Culex restuans* and *Culex pipiens* (Figure 1, 2; G= 14.7, df=9, P=0.10, Table S1), suggesting that the two main vector species interact with a similar set of avian *Plasmodium* hosts. A Mantel test revealed no significant correlation between similarities in relationships with avian *Plasmodium* vectors and *Plasmodium* lineages across avian hosts (r=-0.09, P=0.58), suggesting that host interactions with *Plasmodium* are not structured by the limited (and insignificant) variation in host utilisation by mosquito vectors. This result remained unchanged when considering infections from hatch-year or after hatch-year birds independently (see Supplementary Material, Section 4).

In contrast, relationships between avian host species and *Plasmodium* lineages were strikingly heterogeneous (Table 1; G=411, df= 54, $p<0.001$). Non-metric multidimensional scaling demonstrated relationships between *Plasmodium* lineages, avian hosts, and *Culex* vectors (Figure 2). The ordination split hosts and parasites into two groups. Host species within the superfamily Muscicapoidea (*T. migratorius, Sturnus vulgaris*, *Dumetella carolinensis*) overlap with the parasite lineages CHI02PL,

CHI04PL, CHI07PL, and CHI09PL, while those within the superfamily Passeroidea (*Agelaius phoeniceus*, *Cardinalis cardinals*, *Carpodacus mexicanus*, *Melospiza melodia*, *Molothrus ater*, *Passer domesticus*, *Quiscalus quiscula*) group with *Plasmodium cathermerium*, *Plasmodium elongatum*, and CHI05PL. A Mantel test revealed a positive correlation (Mantel r = 0.58, p=0.006) between phylogenetic similarity as indicated by branch lengths separating host species (Table S2) and the similarity of parasite relationships between host species pairs. The *Plasmodium* assemblage on *Culex* vectors grouped within the Muscicapoidea cluster. CHI02PL, CHI04PL, CHI07PL, and CHI09PL composed 64% of the *Plasmodium* parasites in *Culex* vectors. *P. cathermerium*, *P. elongatum*, and CHI05PL composed 36% of that parasite assemblage.

Pairwise G-tests offered a statistically explicit approach to assessing differences in *Plasmodium* assemblages across hosts and vectors. The tests, summarised in Figure S1, demonstrate that the *Plasmodium* assemblage of *T. migratorius* differed significantly from all other assemblages. This is associated with the high degree of association between *T. migratorius* and 4 of 7 common *Plasmodium* lineages. Seven other pairwise comparisons differed significantly. Five of these pairs compared assemblages of Muscicapoidea and Passeroidea hosts. Excluding *T. migratorius*, all comparisons between host pairs within Musicapoidea or the nine-primaried New World Passeroidea (all Passeroidea host here except *P. domesticus*) were statistically indistinguishable. Interestingly, 8 of 10 comparisons between the *Plasmodium* assemblages on vectors and those of avian hosts exhibited significant differences.

Three separate Monte Carlo simulations, each with a unique set of assumptions (Supplementary Material, Section S3), revealed patterns consistent with the other analyses. The simulations suggest *T. migratorius* have more CHI02PL, CHI04PL, and CHI07PL infections and less *P. elongatum*, *P. cathermerium*, and CHI05PL infections than expected (Supplementary Material, Tables S3a-e). Well-sampled Passeroidea hosts showed the opposite pattern. See Supplementary Material, Section 3 for more information.

Discussion

Our original model of a limiting host-encounter filter for vector-borne parasites hinged on a key assumption: vectors feed on different subsets of hosts and these divergent feeding patterns structure parasite assemblages on hosts. This assumption was not supported by any of our analyses. Feeding patterns of the two dominant avian *Plasmodium* vectors were similar, highly connected, and provided different *Plasmodium* lineages the same relative access across host species. Moreover, the limited variation in the feeding patterns between *Culex restuans* and *Culex pipiens* did not explain variation in *Plasmodium* assemblages across hosts. Our data demonstrate that the feeding patterns of *Culex* mosquitoes in Chicago, IL, do not impose a compartmentalised encounter filter that structures the relationships between *Plasmodium* taxa and common avian host species.

Assemblages of *Plasmodium* parasites on avian host species were heterogeneous despite the similar feeding patterns of the two *Culex* species. This strongly suggests that compatibility issues that exist solely between the host and

parasite structure these *Plasmodium*-bird relationships. This is corroborated by three important results of our analyses. 1) Significant differences exist between the *Plasmodium* assemblage on mosquito vectors and 8 of 10 of the *Plasmodium* assemblages on hosts. In the absence of compartmentalised vector feeding patterns, these differences must arise from differential compatibilities between host and parasite pairs. 2) Monte Carlo simulations demonstrate that the frequency of infections of particular lineages in specific host species deviate from expectations. These comparisons reveal the presence of specific compatibility filters. 3) Both the NMDS ordination and a Mantel test revealed that host relationships with *Plasmodium* parasites are phylogenetically structured in this system. Like other studies (Gilbert and Webb 2007, Pearlman and Jaenike 2003), this suggests that the compatibility filter strengthens with increasing phylogenetic distance.

Specific examples of both strong and porous host compatibility filters were evident within our data. Many hosts had fewer infections of specific *Plasmodium* lineages than expected by random assortment of hosts and parasites or the relative access provided by mosquito vectors. For instance, CHI02PL, CHI04PL, and CHI07PL were absent to rare in Passeroidea hosts despite these lineages making up 64% of the infections in vectors. Perhaps the most striking example of parasite-host incompatibility is the near absence of *P. elongatum* and *P. cathermerium* from *T. migratorius*, despite these parasites being common in *Culex* mosquitoes and the high frequency of contact between *T. migratorius* and these vectors. The apparent cases of incompatibility may arise through two distinct mechanisms. These *Plasmodium* lineages may have high virulence on these host species, and increase the probability of

mortality before sampling (Wilcox *et al.* 2007). Alternatively, these hosts may be resistant to the infection. This could be due to adaptations of the immune system, such as those associated with major histocompatibility complex (Westerdahl *et al.* 2005, Bonneaud *et al.* 2006, Loiseau *et al.* 2011) or host cell surface proteins (Cowman and Crabb 2006), the lack of necessary machinery of the parasite to invade and persist in certain hosts, or both. Palinauskas *et al.* 2008 demonstrated that experimentally challenged host species differed in their level of resistance toward *Plasmodium relictum*. Ultimately, experimental infection studies like this are necessary to discriminate between these hypotheses.

In addition, some *Plasmodium* lineages were more frequent in specific hosts than expected. *P. cathermerium* and *P. elongatum* occurred more frequently in some Passeroidea hosts. CHI05PL was recovered disproportionately from *P. domesticus*. However, the most obvious example of this is the frequent recovery of CHI02PL, CHI04PL, and CHI07PL from *T. migratorius*. These parasites were largely restricted to *T. migratorius,* and parasitised this host at rates that exceeded expectations generated by random association or the vector-imposed encounter rate. Indeed, our analyses suggest that CHI02PL, CHI04PL, and CHI07PL may be specialised on *T. migratorius*. Specialisation on *T. migratorius* may not be coincidental. This host species accounts for more than 60% of the blood meals of both *Culex* vector species making it the most encountered host in the community for mosquito-borne *Plasmodium* parasites. The high probability of encounter for these *Plasmodium* parasites with *T. migratorius* likely mitigates a primary cost of specialisation: the failure to find optimal hosts because they are infrequent in a multihost community

(Hellgren *et al.* 2009).

Expansions in host range can result when changes in vector-host contact rates introduce parasites to novel hosts (Graham *et al.* 2009). However, numerous studies have revealed an important interplay between host compatibility and the encounter rate in driving pathogen transmission dynamics over time (Kilpatrick *et al.* 2006a), space (Allan *et al.* 2010), and between ecological communities that differ in structure (Simpson *et al.* 2012, LoGiudice *et al.* 2003). Indeed, host range expansions also depend on the compatibility of novel hosts toward those parasites, and will not proceed if new host-parasite combinations are incompatible. Traits that influence host compatibility, and its constituent properties of host susceptibility, parasite infectivity, and the virulence of infection, evolve over time (Decaestecker *et al.* 2007, Woodward *et al.* 2005). In the West Indies, the same suite of avian hosts and malaria parasites assemble into different patterns of relationships across island replicates (Fallon *et al.* 2003,2005, Ricklefs *et al.* 2011), and there is some evidence that these differences can arise over short time periods (Fallon *et al.* 2004). If host compatibility issues outweigh heterogeneity in the encounter rate in structuring these parasite-host relationships, such idiosyncratic patterns observed in the West Indies and elsewhere may suggest that compatibility mechanisms are highly labile, even when parasites with complex life cycles are involved.

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Tables and Figures

Table 1. Number of *Plasmodium* infections of specific lineages across all 10 avian hosts and *Culex* mosquito vectors. $MLE_{Cx.}$ is a bias-corrected maximum-likelihood estimate of the number of infected mosquitoes per 1000 individuals for each *Plasmodium* lineage. Upper and lower 95% confidence limits are shown within parentheses. Abbreviations for host species include the first letter of the genus, and the first two letters of the species name respectively. APH= *Agelaius phoeniceus* (Red-winged Blackbird); CCA=*Cardinalis cardinalis,* (Northern Cardinal); CME=*Carpodacus mexicanus*, (House Finch); DCA= *Dumetella carolinensis,* (Gray Catbird); MAT= *Molothrus ater*, (Brown-headed Cowbird); MME= *Melospiza melodia*, (Song Sparrow); PDO= *Passer domesticus,* (House Sparrow); QQU=*Quiscalus quiscula*, (Common Grackle); SVU= *Sturnus vulgaris,* (European Starling); TMI= *Turdus migratorius* (American Robin).

Fig. 1. A tripartite interaction network demonstrating the relationships between avian hosts, *Culex* vectors, and *Plasmodium* parasites in Chicago, IL, USA. The topology of the host phylogenetic tree was based on a maximum likelihood analysis of RAG1 (see Supplementary Material). Connections between host and parasite, and host and vector are based on the parasite screening results presented here, and vector blood meal

analyses presented in Hamer *et al.* 2009. Connections between mosquito vectors and parasites denoted by solid lines are based on published accounts of vectorial capacity (summarised in Valkiūnas 2005) or documented infections that were naturally acquired in those mosquito species (Kimura *et al.* 2010). Connections denoted by dashed lines are not reported in either Valkiūnas 2005 or Kimura *et al.* 2010, but instead are inferred from data presented here where exact species-level interactions cannot be determined due to mixed *Culex* mosquito pools.

Fig. 2. Plot demonstrating the results of a 3-dimensional nonmetric multidimensional scaling ordination of parasite-host relationships. The font size of the text is directly proportional to the value in the third dimension. The three letter abbreviations for hosts include the first letter of the genus name and first two letters of the species name in that order. "Vector" represents the assemblage of parasites found in positive *Culex* pools. The proximity of hosts in this 3-dimensional ordination space demonstrates similarity in their parasite assemblages. The relative positions of the parasites in this 3-dimensional ordination space graphically demonstrate the composition of these assemblages. *CxP* and *CxR* represent the distribution of blood meals for *Culex pipiens* and *Culex restuans*, respectively. These points were calculated as $\Sigma\, p_i NMDS1_i + p_i NMDS2_i + p_i NMDS3_i$ where p_i is the proportion of blood meals of host species *i* for a mosquito species and $NMDSx_i$ is the *x*-dimension NMDS score of host species *i*. These points are centrally located between all potential host species and overlap significantly, suggesting broad and similar feeding patterns between the two *Culex* species

Hosts	*Plasmodium* Parasites							
	CHI02PL	CHI04PL	CHI07PL	CHI09PL	P.CATH	P.ELON	CHI05PL	TOTAL
APH	0	0	0	0	4	2	1	7
CCA	0	0	0	0	26	24	2	52
CME	0	0	0	0	4	12	0	16
DCA	0	2	4	0	4	10	0	20
MAT	0	0	0	0	3	7	0	10
MME	0	0	0	0	11	6	3	20
PDO	0	0	2	1	56	17	20	96
SVU	0	0	6	0	7	0	0	13
TMI	35	23	144	9	9	5	2	227
QQU	0	0	0	0	10	2	0	12
TOTAL	35	25	156	10	134	85	28	
Vectors								
CULEX	14	37	29	2	32	12	2	128
$MLE_{Cx.}$	2.3 (1.3, 3.8)	6.35 (4.6, 8.7)	4.7 (3.2, 6.8)	0.32 (0.06, 1.1)	5.3 (3.6, 7.4)	2.0 (1.1, 3.3)	0.32 (0.06, 1.1)	

Table 1.

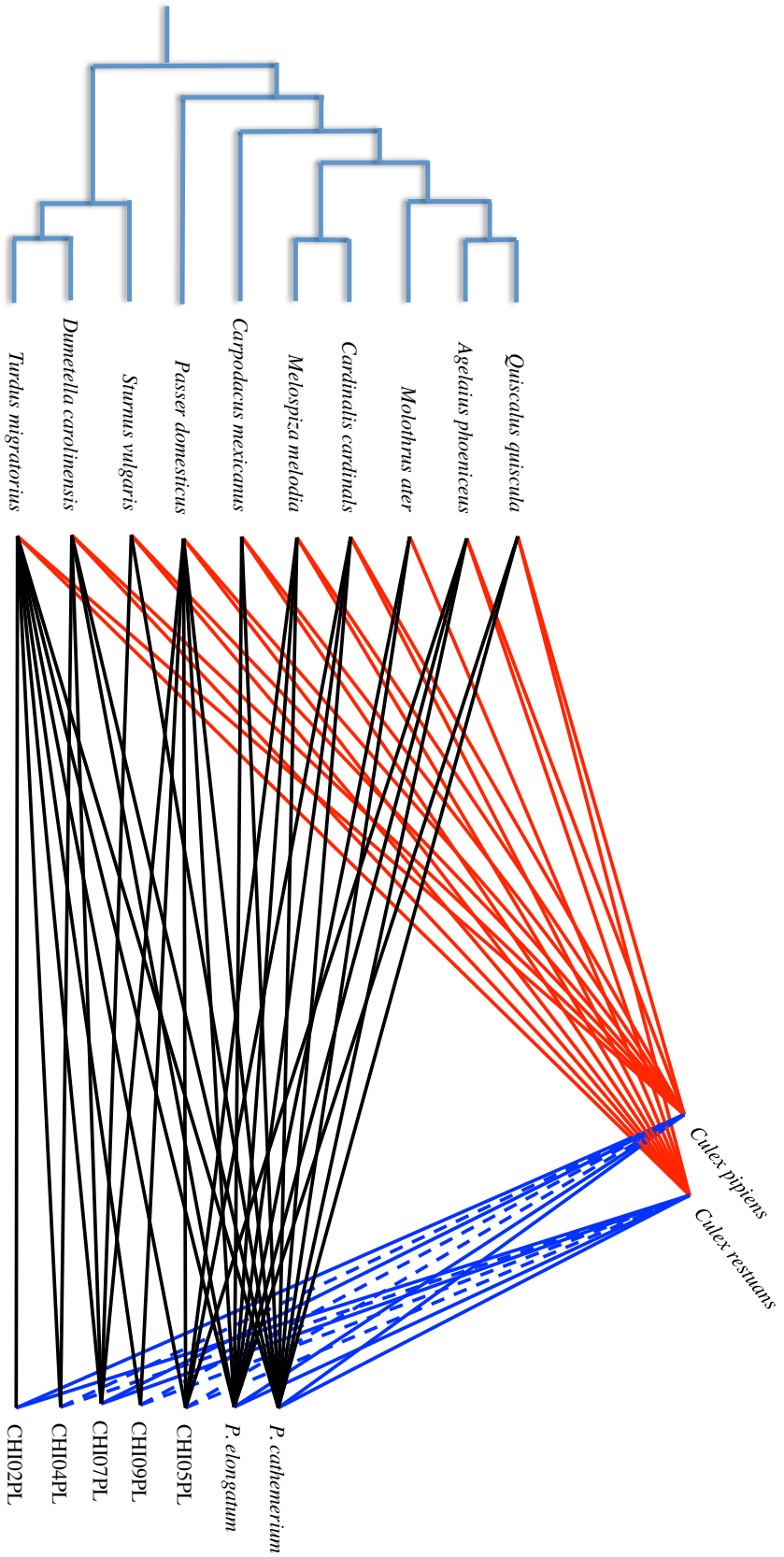
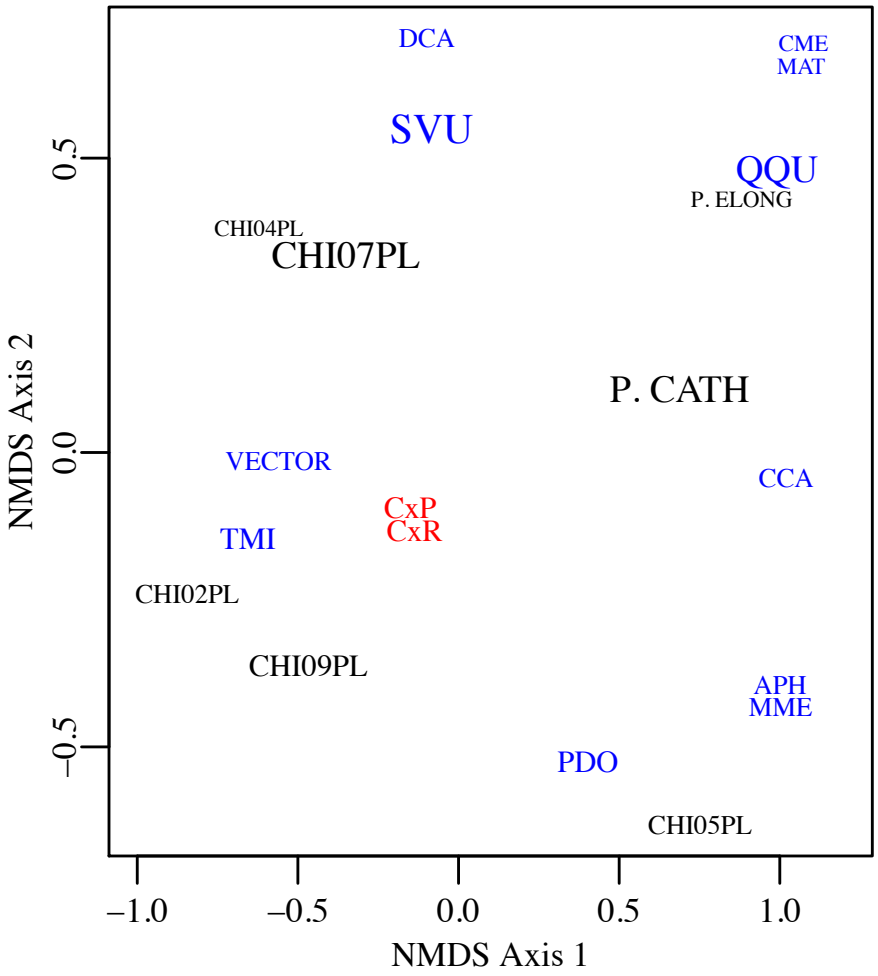

Quiscalus quiscula
Agelaius phoeniceus
Molothrus ater
Cardinalis cardinalis
Melospiza melodia
Carpodacus mexicanus
Passer domesticus
Sturnus vulgaris
Dumetella carolinensis
Turdus migratorius
Culex pipiens
Culex restuans
P. cathemerium
P. elongatum
CHI05PL
CHI09PL
CHI07PL
CHI04PL
CHI02PL

Figure 1.

Figure 2.


DCA
CME
MAT
SVU
QQU
P. ELONG
CHI04PL
CHI07PL
P. CATH
VECTOR
CCA
CxP
CxR
TMI
CHI02PL
CHI09PL
APH
MME
PDO
CHI05PL
0.5
0.0
−0.5
NMDS Axis 2
−1.0
−0.5
0.0
0.5
1.0
NMDS Axis 1

Supplemental Information

Section 1. Supplementary figures and data tables

	TMI	SVU	DCA	QQU	APH	MAT	MME	CME	CCA	PDO
SVU	19.7									
DCA	43.1	9.2								
QQU	44.3	5.5	8.3							
APH	30.0	5.1	4.2	1.5						
MAT	38.9	10.4	1.9	4.6	2.1					
MME	71.3	10.1	8.5	1.8	0.9	3.2				
CME	61.2	14.9	3.2	7.1	4.0	0.3	5.4			
CCA	166.8	19.9	12.8	4.9	5.0	3.7	3.8	4.1		
PDO	249.1	18.2	25.2	4.7	4.5	10.7	2.9	16.5	14.4	
Culex	84.3	8.5	14.9	13.4	9.2	15.9	25.7	27.3	64.7	100.1

Muscicapoidea

Passeroidea

Figure S1. A grid summarising pairwise comparisons of parasite assemblages on avian hosts and the mosquito vectors in Chicago, IL, USA. The numbers within grid cells are G-statistics. Shaded grid cells denote statistically different comparisons ($p<0.05$; for all comparisons df=6, G-crit=12.6). The parasite community on *Turdus migratorius* (TMI) is distinct from all other hosts. Parasite communities on other hosts within Muscicapoidea (DCA and SVU) are distinct from some communities on hosts within Passeroidea. Moreover, the parasite community within vectors is distinct from those in most hosts.

Table S1. Contingency table of *Culex* blood meals in Chicago, IL across the 10 common avian host species analysed. Whole digits represent the number of blood meals of an avian host species retrieved from a particular *Culex* species. The number within the parentheses represents the proportion of blood meals derived from a particular host species of the total number of blood meals for each *Culex* species.

	Culex pipiens	*Culex restuans*
Agelaius phoeniceus	2 (0.004)	4 (0.018)
Cardinalis cardinals	42 (0.090)	25 (0.110)
Carpodacus mexicanus	37 (0.079)	8 (0.035)
Dumetella carolinensis	2 (0.004)	2 (0.009)
Melospiza melodia	2 (0.004)	1 (0.004)
Molothrus ater	0	2 (0.009)
Passer domesticus	77 (0.165)	37 (0.163)
Quiscalus quiscula	3 (0.006)	2 (0.009)
Sturnus vulgaris	12 (0.026)	12 (0.053)
Turdus migratorius	289 (0.620)	134 (0.590)

Section 2. Recombination activating gene 1 (RAG1) phylogeny

Phylogenetic distances among hosts were estimated by sequencing and aligning a 656-bp fragment of the recombination activating gene 1 (RAG1). RAG-1 was amplified with primers RAG-1F (5'GCA AKA ATA YAC ATC TCA GYACCA MG 3') and RAG-1R (5' GCT GYA TCA TAT CGR AAT CTC TTY GC 3'). PCR reactions consisted of 1X buffer, 200 nM of each dNTP, 2 mM MgCl2, 0.02% BSA, 200 nM of each primer, and 0.5 units of TaKaRa Taq^{TM} (TaKaRa Bio Inc., Shiga, Japan). The PCR involved an initial denaturing period at 94°C for 4 min, 35 cycles of 94°C for 30 s, 55°C for 30 s, 72°C for 1 min, and a final extension step at 72°C for 3 min. A maximum likelihood gene tree was constructed with a GTR + γ model (gamma=0.23) using the PHYML plug-in in the program Geneious. *Gallus gallus* (AF143730), *Meliphaga analoga* (AY057003), and *Formicarius colma* (AY056993) were included as outgroups. GenBank accession numbers for RAG-1 sequences of all

hosts are as follows: TMI (KC789829), MAT (KC789831), QQU (KC789830), SVU (AY057032), DCA (AY319981), CME (EU165349), PDO (EF568263), CCA (AY056982), APH (KC789833), and MME(KC789832). Patristic distances were extracted from the tree and used in subsequent analyses (Table S2).

Table S2. Table of the patristic distances of hosts based on a maximum likelihood analysis of a 656-bp fragment of the recombination-activating gene 1 (RAG1). The species abbreviation code includes the first letter of the genus name and the first two letters of the species name.

	TMI	MAT	QQU	SVU	DCA	CME	PDO	CCA	APH
MAT	0.071								
QQU	0.070	0.014							
SVU	0.039	0.062	0.060						
DCA	0.041	0.068	0.067	0.036					
CME	0.059	0.035	0.033	0.050	0.056				
PDO	0.054	0.037	0.035	0.045	0.051	0.024			
CCA	0.064	0.036	0.034	0.055	0.061	0.027	0.029		
APH	0.067	0.011	0.006	0.057	0.064	0.030	0.032	0.031	
MME	0.063	0.034	0.034	0.054	0.060	0.027	0.029	0.018	0.030

Section 3. Monte Carlo simulation of parasite distributions across hosts

Set 1. Monte Carlo simulations of the distribution of Plasmodium *parasites across avian hosts based on their cumulative frequency in the sample.*

For each run of this simulation, host individuals of a given species were assigned to *Plasmodium* infections based on the proportion of a specific *Plasmodium* lineage in the sample (Table 1). The original number of infections per host species was maintained in each run of the simulation. 100,000 runs were performed. This procedure was repeated for each host species.

Table S3a summarises the results. The actual number of infections for 24 of 70 possible host-parasite combinations was outside the 5% confidence limits of the

simulated distribution. CHI09PL, *Agelaius phoeniceus*, and *Sturnus vulgaris* did not demonstrate any deviations from the expected number of infections. In general, the parasite lineages CHI02PL and CHI07PL were less abundant than expected on hosts within the superfamily Passeroidea. This was especially apparent for the well-sampled *Passer domesticus* and *Cardinalis cardinalis,* and for the CHI07PL across many hosts. In addition, CHI02PL, CHI04PL, and CHI07PL were more abundant on *Turdus migratorius* than expected. *P. cathermerium* and *P. elongatum*, showed the opposite pattern, being overly abundant across many Passeroidea hosts and nearly absent on *Turdus migratorius*. CHI05PL was overly abundant on *Passer domesticus*, and less abundant on *Turdus migratorius.*

This analysis generally demonstrates how the distribution of *Plasmodium* parasites across host species analysed here differs from random expectations based on the frequency in which both parasites and host species were sampled.

Set 2. Monte Carlo simulations of the distribution of parasites across hosts based on the actual proportion of parasites in Culex *mosquito vectors.*

For each run of this simulation, host individuals of a given species were assigned to *Plasmodium* lineages based on the proportion of the *Plasmodium* lineage within the sample of infected vectors (Table 1). The original number of infections per host species was maintained in each run of the simulation. 100,000 runs were performed. This procedure was repeated for each host species.

Table S3b summarises the results. The actual number of infections for 31 of 70 possible host-parasite combinations was outside the 5% confidence limits of the simulated distribution. *Agelaius phoeniceus* did not demonstrate any deviations from

expected number of infections. General patterns were similar to Set 1, with CHI02PL and CHI07PL being more common on *T. migratorius* and less common of Passeroidea hosts, and *P. elongatum*, *P. cathemerium*, and CHI05PL being less common on *T. migratorius* and more common of Passeroidea hosts. Interestingly, most hosts had fewer CHI04PL infections than expected. This is associated with an unexpectedly high proportion of CHI04PL infections in *Culex* vectors.

If the two species of *Culex* vectors feed on hosts at equivalent rates, each parasite has the same relative access to hosts independent of its vector. Assuming that host compatibility issues were not present, this would suggest that hosts and vectors would have the same relative proportions of each parasite. Both this simulation and the G-test comparing the parasite assemblage in vectors to those of specific host species (Figure S1) show this is not the case. The results suggest that a strong host compatibility filter restricts the distribution of parasites across hosts. Unlike the G-test however, this approach provides a statistically explicit way of identifying host-parasite pairs that depart from random. Once again, this simulation highlights the lack of *P. elongatum* and *P. cathemerium* infections in *T. migratorius.*

Set 3. Monte Carlo simulations of the distribution of Plasmodium *parasites across hosts based on the frequency of the parasites and mosquito vector-feeding probabilities on each host.*

For each run of this simulation, *Plasmodium* infections were assigned to hosts of a given species based on the proportion of *Culex* blood meals derived from that species. Here, the proportion of blood meals is assumed to represent the probability that an infectious mosquito will bite a particular host species. Because

both *Culex* species had statistically indistinguishable feeding patterns, they were combined to estimate this probability. The original number of infections of each parasite lineage in the sample was maintained in each run of the simulation. 100,000 runs were performed. This procedure was repeated for each *Plasmodium* lineage.

Because this procedure assigns *Plasmodium* lineages to hosts based on the feeding probabilities of vectors, it does not explicitly account for host abundance. Instead, we assume that the prevalence of each lineage is constant across the simulation and the actual sample. Thus, the expected values and the confidence limits generated by the simulation express the number of parasites of a specific lineage that should be recovered from each host species given that 1) the same number of birds (N=1596) were resampled and 2) mosquito biting probabilities determine *Plasmodium* host range. Therefore, the actual number of infections per host species is only comparable to these expected values and their confidence limits if host species were sampled commensurate to their relative abundance and availability to host-seeking mosquitoes. We use both point counts with distance sampling and mist-netting capture data to estimate the relative abundance of each species in the community (Table S3c). A description of the methods on the point count surveys can be found in Hamer *et al.* 2009. The relative abundance of bird populations can be difficult to estimate as different techniques have inherent biases. Ground-level mist-nets may vary in their ability to capture birds of different sizes, and may be biased against those that occupy the canopy (although the relatively minor vertical stratification of urban-suburban habitat makes this less of a concern for our study). Point counts may miss individuals of cryptic species that are less

conspicuous. Thus, we averaged the relative proportions of each species across both methods to mitigate the inherent biases of each technique by itself.

The proportion of each species in the actual sample was highly correlated with the average proportion of each in the community (Figure S2, R^2=0.89, $p<0.0001$). However, the slope of the regression line (0.71) demonstrated that the values of each proportion were not equal. Therefore, we rescaled the results of the initial simulation (summarized in Table S3d) by converting the expected confidence limits and the actual values into prevalence. We divide the expected confidence limits by the number of individuals of each species that should exist in a community of 1596 birds sampled without bias (ie. the average proportion of a species in the community * 1596). We divide the actual values by the actual number of individuals sampled per host species. Rescaled values are summarized in Table S3e.

This simulation attempts to control for a mosquito-imposed encounter rate. Thus, cases in which observed values deviate from the range of expected values might highlight specific cases in which a host compatibility filter is operating. This simulation is slightly more conservative than others presented here. The prevalences of 22 of 70 possible host-parasite combinations were outside the 5% confidence limits of the simulated distribution. However, the same major pattern evident throughout our analysis maintains. CHI07PL, CHI02PL, and CHI04PL (though marginally so for the later) are more prevalent on *T. migratorius* than expected, while CHI05PL, *P. cathemerium*, and *P. elongatum* are less prevalent on *T. migratorius* than expected. The prevalences of *P. cathemerium*, and *P. elongatum* are equal to or exceed the expected prevalences on most Passeroidea hosts (although

C. mexicanus and *P. cathemerium* is an interesting exception). CHI07PL and CHI02PL are nearly absent from Passeroidea host, and are less prevalent on well-sampled Passeroidea hosts than expected. CHI05PL is more prevalent on 3 Passeroidea hosts than expected, most notably on *P. domesticus*.

	CHI02PL			CHI04PL			CHI07PL			CHI09PL			P.CATH			P.ELON			CHI05PL		
	EV	CL	AV	EV	CL	AV	EV	CL	AV	EV	CL	AV	EV	CL	AV	EV	CL	AV	EV	CL	AV
APH	0.52	0,2	0	0.37	0.2	0	2.31	0,5	0	0.15	0,1	0	1.99	0,4	4	1.25	0,3	2	0.42	0,2	1
CCA	3.84	1,8	0	2.75	0,6	0	17.16	11,24	0	1.11	0,4	0	14.74	9,21	26	9.33	4,15	24	3.07	0,7	2
CME	1.18	0,4	0	0.85	0,3	0	5.29	2,9	0	0.34	0,2	0	4.53	1,8	4	2.88	0,6	12	0.95	0,3	0
DCA	1.49	0,4	0	1.06	0,3	2	6.60	3,11	4	0.42	0,2	0	5.66	2,10	4	3.59	1,7	10	1.18	0,4	0
MAT	0.74	0,3	0	0.53	0,2	0	3.30	1,6	0	0.21	0,1	0	2.83	0,6	3	1.79	0,4	7	0.60	0,2	0
MME	1.49	0,4	0	1.06	0,3	0	6.59	3,11	0	0.42	0,2	0	5.67	2,10	11	3.60	1,7	6	1.19	0,4	3
PDO	7.12	3,13	0	5.07	1,10	0	31.65	23,41	2	2.03	0,5	1	27.22	19,36	56	17.22	10,25	17	5.68	2,11	20
SVU	0.96	0,3	0	0.69	0,3	0	4.28	1,8	6	0.28	0,2	0	3.69	1,7	7	2.34	0,5	0	0.77	0,3	0
TMI	16.81	10,25	35	11.98	6,19	23	74.88	61,89	144	4.79	1,9	9	64.32	51,78	9	40.77	30,52	5	13.46	7,21	2
QQU	0.89	0,3	0	0.64	0,2	0	3.95	1,7	0	0.25	0,2	0	3.41	1,7	10	2.15	0,5	2	0.70	0,3	0

Table S3a. Results of Monte Carlo simulations (Set 1) of the distribution of *Plasmodium* parasites across avian hosts based their cumulative frequency in the sample. EV is the expected value and represents the mean simulated value across the 100,000 runs. CL shows the 95% confidence limits based on the 100,000 runs in the simulation. AV is the actual number of infections observed. Highlighted cells represent host-parasite pairs in which the actual value of infections lies outside the 95% confidence limits of the simulation. Abbreviations for host species include the first letter of the genus, and the first two letters of the species name respectively.

	CHI02PL			CHI04PL			CHI07PL			CHI09PL			P.CATH			P.ELON			CHI05PL		
	EV	CL	AV	EV	CL	AV	EV	CL	AV	EV	CL	AV	EV	CL	AV	EV	CL	AV	EV	CL	AV
APH	0.76	0,3	0	2.02	0,4	0	1.59	0,4	0	0.11	0,1	0	1.75	0,4	4	0.65	0,2	2	0.11	0,1	1
CCA	5.69	2,10	0	15.03	9,22	0	11.78	6,18	0	0.81	0,3	0	13.01	7,19	26	4.88	1,9	24	0.81	0,3	2
CME	1.75	0,4	0	4.62	1,8	0	3.64	1,7	0	0.25	0,2	0	4.00	1,8	4	1.50	0,4	12	0.25	0,2	0
DCA	2.18	0,5	0	5.79	2,10	2	4.53	1,8	4	0.31	0,2	0	5.00	2,9	4	1.88	0,5	10	0.31	0,2	0
MAT	1.10	0,3	0	2.89	0,5	0	2.27	0,5	0	0.16	0,1	0	2.50	0,5	3	0.94	0,3	7	0.16	0,1	0
MME	2.19	0,5	0	5.78	2,10	0	4.53	1,8	0	0.31	0,2	0	5.00	2,9	11	1.87	0,5	6	0.31	0,2	3
PDO	10.50	5,17	0	27.72	19,37	0	21.76	14,30	2	1.50	0,4	1	24.01	16,33	56	9.00	4,15	17	1.50	0,4	20
SVU	1.42	0,4	0	3.76	1,7	0	2.94	0,6	6	0.20	0,1	0	3.26	1,6	7	1.22	0,4	0	0.20	0,1	0
TMI	24.83	16,34	35	65.60	52,79	23	51.45	39,64	144	3.55	0,8	9	56.78	44,70	9	21.26	13,30	5	3.55	0,8	2
QQU	1.31	0,4	0	3.47	1,7	0	2.73	0,6	0	0.19	0,1	0	3.00	0,6	10	1.12	0,3	2	0.19	0,1	0

Table S3b. Results of Monte Carlo simulations (Set 2) of the distribution of parasites across hosts based on the actual proportion of parasites in *Culex* mosquito vectors. EV is the expected value and represents the mean simulated value across the 100,000 runs. CL shows the 95% confidence limits based on the 100,000 runs in the simulation. AV is the actual number of infections observed. Highlighted cells represent host-parasite pairs in which the actual value of infections lies outside the 95% confidence limits of the simulation. Abbreviations for host species include the first letter of the genus, and the first two letters of the species name respectively.

Table S3c. Table demonstrating the number of individuals sampled and screened for *Plasmodium* parasites, the proportion of this sample, and the proportion in the avian community as measured through point surveys with distance sampling methods (Hamer *et al*. 2009) and mist-net captures for each host species.

Host Species	Number Sampled	Proportion of the sample	Proportion in community (point counts)	Proportion in community (net captures)
APH	55	0.034	0.054	0.036
CCA	122	0.076	0.017	0.064
CME	79	0.049	0.013	0.032
DCA	151	0.095	0.006	0.092
MAT	20	0.013	0.003	0.013
MME	72	0.045	0.002	0.044
PDO	545	0.341	0.526	0.452
SVU	66	0.041	0.068	0.028
TMI	435	0.273	0.242	0.219
QQU	51	0.032	0.069	0.020
Sum	1596	1	1	1

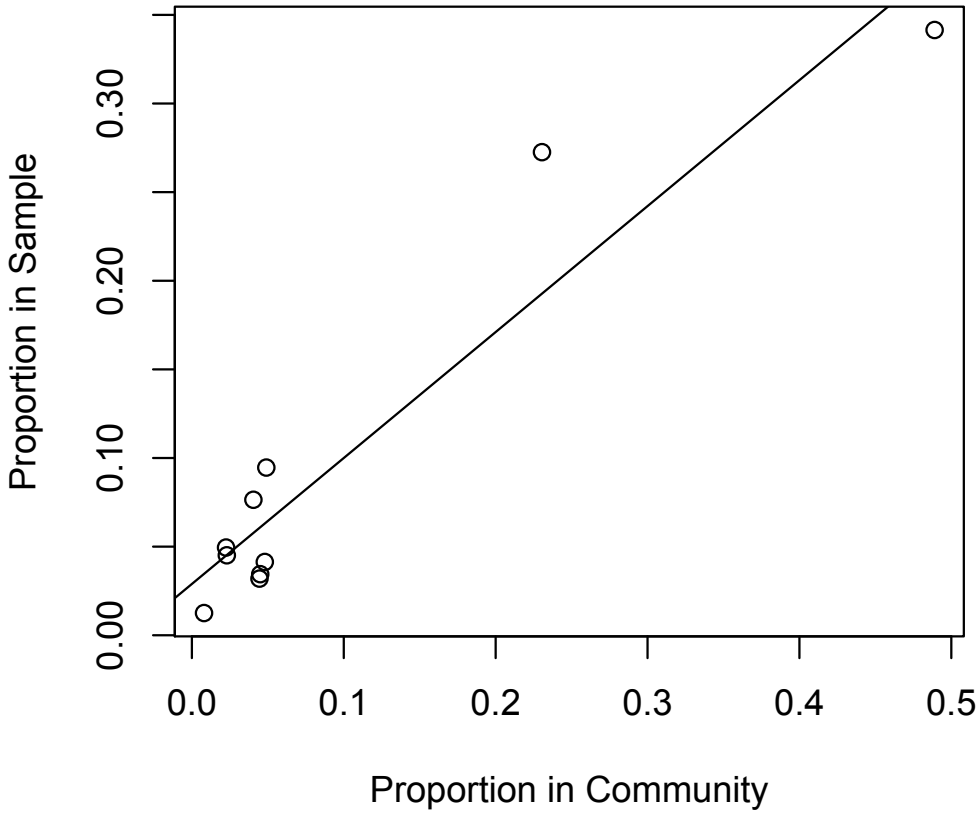


Figure S2. Plot of the proportion of a host species in the sample regressed against the estimated proportion in the community. The community proportion is estimated by averaging host species proportions based on point counts and mist net captures (see Table 3c).

		CHI02PL	CHI04PL	CHI07PL	CHI09PL	P.CATH	P.ELON	CHI05PL
APH	EV	0.31	0.22	1.35	0.09	1.16	0.74	0.24
	CL	0,2	0,1	0,4	0,1	0,4	0,3	0,1
	AV	0	0	0	0	4	2	1
CCA	EV	3.39	2.41	15.08	0.97	12.94	8.22	2.7
	CL	0,7	0,6	8,23	0,3	7,20	3,14	0,6
	AV	0	0	0	0	26	24	2
CME	EV	2.28	1.63	10.12	0.65	8.7	5.52	1.82
	CL	0,5	0,4	5,16	0,2	4,15	2,10	0,5
	AV	0	0	0	0	4	12	0
DCA	EV	0.20	0.14	0.9	0.06	0.78	0.49	0.16
	CL	0,1	0,1	0,2	0,1	0,3	0,2	0,1
	AV	0	2	4	0	4	10	0
MAT	EV	0.10	0.07	0.45	0.03	0.39	0.24	0.08
	CL	0,1	0,1	0,2	0,1	0,2	0,2	0,1
	AV	0	0	0	0	3	7	0
MME	EV	0.15	0.11	0.67	0.04	0.58	0.37	0.12
	CL	0,1	0,1	0,3	0,1	0,2	0,2	0,1
	AV	0	0	0	0	11	6	3
PDO	EV	5.76	4.11	25.66	1.64	22.04	13.99	4.61
	CL	2,10	1,8	17,35	0,4	14,31	8,21	1,9
	AV	0	0	2	1	56	17	20
SVU	EV	1.21	0.86	5.41	0.35	4.64	2.95	0.97
	CL	0,4	0,3	1,10	0,2	1,9	0,7	0,3
	AV	0	0	6	0	7	0	0
TMI	EV	21.35	15.27	95.24	6.1	81.82	51.87	17.09
	CL	16,27	10,20	83,107	3,9	71,93	43,61	12,22
	AV	35	23	144	9	9	5	2
QQU	EV	0.25	0.18	1.13	0.07	0.96	0.62	0.2
	CL	0,2	0,1	0,4	0,1	0,3	0,2	0,1
	AV	0	0	0	0	10	2	0

Table S3d. Results of Monte Carlo simulations (Set 3) of the distribution of parasites across hosts based on the frequency of the parasites and mosquito vector-feeding probabilities on each host. EV is the expected value and represents the mean simulated value across the 100,000 runs. CL shows the 95% confidence limits based on the 100,000 runs in the simulation. AV is the actual number of infections observed. Abbreviations for host species include the first letter of the genus, and the first two letters of the species name respectively.

Table S3e. Rescaled lower and upper 95% confidence limits (LCL/UCL, respectively) from Set 3 of the Monte Carlo simulations. Confidence limits and the actual values are rescaled by dividing the expected number of individuals per host species (average proportion of the species in the community * 1598) and the number of host actually sampled per species, respectively (see Tables S3c & S3d). Thus, cell values represent rescaled prevalences to mitigate bias in sampling effort. Host identities are in the upper-left corner for each sub-table.

APH	rescaled LCL	rescaled UCL	rescaled AV
CHI02PL	0.000	0.028	0.000
CHI04PL	0.000	0.014	0.000
CHI07PL	0.000	0.056	0.000
CHI09PL	0.000	0.014	0.000
P.CATH	0.000	0.056	0.073
P.ELON	0.000	0.042	0.036
CHI05PL	0.000	0.014	0.018

CCA	rescaled LCL	rescaled UCL	rescaled AV
CHI02PL	0.000	0.108	0.000
CHI04PL	0.000	0.093	0.000
CHI07PL	0.124	0.356	0.000
CHI09PL	0.000	0.046	0.000
P.CATH	0.108	0.309	0.213
P.ELON	0.046	0.217	0.197
CHI05PL	0.000	0.093	0.016

CME	rescaled LCL	rescaled UCL	rescaled AV
CHI02PL	0.000	0.139	0.000
CHI04PL	0.000	0.111	0.000
CHI07PL	0.139	0.446	0.000
CHI09PL	0.000	0.056	0.000
P.CATH	0.111	0.418	0.051
P.ELON	0.056	0.278	0.152
CHI05PL	0.000	0.139	0.000

DCA	rescaled LCL	rescaled UCL	rescaled AV
CHI02PL	0.000	0.013	0.000
CHI04PL	0.000	0.013	0.013
CHI07PL	0.000	0.026	0.026
CHI09PL	0.000	0.013	0.000
P.CATH	0.000	0.038	0.026
P.ELON	0.000	0.026	0.066
CHI05PL	0.000	0.013	0.000

MAT	rescaled LCL	rescaled UCL	rescaled AV
CHI02PL	0.000	0.078	0.000
CHI04PL	0.000	0.078	0.000
CHI07PL	0.000	0.157	0.000
CHI09PL	0.000	0.078	0.000
P.CATH	0.000	0.157	0.150
P.ELON	0.000	0.157	0.350
CHI05PL	0.000	0.078	0.000

MME	rescaled LCL	rescaled UCL	rescaled AV
CHI02PL	0.000	0.027	0.000
CHI04PL	0.000	0.027	0.000
CHI07PL	0.000	0.082	0.000
CHI09PL	0.000	0.027	0.000
P.CATH	0.000	0.054	0.153
P.ELON	0.000	0.054	0.083
CHI05PL	0.000	0.027	0.042

PDO	rescaled LCL	rescaled UCL	rescaled AV
CHI02PL	0.003	0.013	0.000
CHI04PL	0.001	0.010	0.000
CHI07PL	0.022	0.045	0.004
CHI09PL	0.000	0.005	0.002
P.CATH	0.018	0.040	0.103
P.ELON	0.010	0.027	0.031
CHI05PL	0.001	0.012	0.037

SVU	rescaled LCL	rescaled UCL	rescaled AV
CHI02PL	0.000	0.052	0.000
CHI04PL	0.000	0.039	0.000
CHI07PL	0.013	0.131	0.091
CHI09PL	0.000	0.026	0.000
P.CATH	0.013	0.117	0.106
P.ELON	0.000	0.091	0.000
CHI05PL	0.000	0.039	0.000

TMI	rescaled LCL	rescaled UCL	rescaled AV
CHI02PL	0.043	0.073	0.080
CHI04PL	0.027	0.054	0.053
CHI07PL	0.226	0.291	0.331
CHI09PL	0.008	0.024	0.021
P.CATH	0.193	0.253	0.021
P.ELON	0.117	0.166	0.011
CHI05PL	0.033	0.060	0.005

QQU	rescaled LCL	rescaled UCL	rescaled AV
CHI02PL	0.000	0.028	0.000
CHI04PL	0.000	0.014	0.000
CHI07PL	0.000	0.056	0.000
CHI09PL	0.000	0.014	0.000
P.CATH	0.000	0.042	0.196
P.ELON	0.000	0.028	0.039
CHI05PL	0.000	0.014	0.000

Section 4. Does host age influence the patterns observed here?

Parasites can be heterogeneously distributed across age classes. Hosts of different ages have variable levels of exposure, with older hosts having an increase exposure often to a wider range of parasites. Moreover, in the case of avian malaria parasites, infections acquired early in life may remain chronic for long-periods of time, even for the duration of the host's life (Valkiunas 2005). Here, we ask whether heterogeneity in parasite-host interactions exists across age classes and influences some of the patterns we report.

The main dataset (presented in Table 1) was divided across two host age classes: hatch year birds (HY), which have only been exposed to one transmission season, and after hatch-year birds (AHY), which have been exposed to more than one transmission season. The resulting datasets are presented in Tables S4a-b. The structure of distance matrices that summarise beta-similarities in the parasite assemblages among hosts of AHY and HY birds were compared by a Mantel test with 10,000 permutations. *Agelaius phoeniceus* was excluded from this analysis because only two HY individuals were sampled and neither had a *Plasmodium* infection. The two matrices were highly correlated (Mantel r = 0.71, p=0.0001) suggesting that differences in the parasite interactions among species are similar between AHY and HY individuals. The same set of Mantel tests presented in the main text was performed on the distance matrices composed of either AHY or HY birds. Beta similarities in the relationship with *Plasmodium* lineages and avian *Plasmodium* vectors across avian hosts were not correlated for both AHY and HY birds (Mantel r = -0.22, -0.09, p=0.88, 0.60; respectively). However, beta-similarities in *Plasmodium*

relationships were correlated with phylogenetic similarity (Mantel r = 0.55, 0.46, p=0.026, 0.017; respectively). Age does not appear to influence the main patterns presented here, namely that 1) relationships with mosquitoes do not limit the distribution of parasites across hosts and 2) phylogenetically related hosts have more similar relationships with parasites.

Table S4a. Number of *Plasmodium* infections of specific lineages across hatch-year (juvenile) individuals of all 10 avian hosts. Abbreviations for host species include the first letter of the genus, and the first two letters of the species name respectively.

Hosts	*Plasmodium* Parasites							
	CHI02PL	CHI04PL	CHI07PL	CHI09PL	P.CATH	P.ELON	CHI05PL	TOTAL
CCA	0	0	0	0	16	15	1	32
CME*	0	0	0	0	2	7	0	9
DCA	0	2	2	0	2	4	0	10
MAT*	0	0	0	0	1	3	0	4
MME	0	0	0	0	3	4	3	10
PDO	0	0	1	0	34	8	7	50
SVU	0	0	2	0	6	0	0	8
TMI	8	15	43	6	9	4	1	86
QQU	0	0	0	0	5	1	0	6
TOTAL	8	17	48	6	78	46	12	

Table S4b. Number of *Plasmodium* infections of specific lineages across after hatch-year (adult) individuals of all 10 avian hosts. Abbreviations for host species include the first letter of the genus, and the first two letters of the species name respectively.

Hosts	*Plasmodium* Parasites							
	CHI02PL	CHI04PL	CHI07PL	CHI09PL	P.CATH	P.ELON	CHI05PL	TOTAL
CCA	0	0	0	0	10	9	1	20
CME*	0	0	0	0	1	5	0	6
DCA	0	0	2	0	2	6	0	10
MAT*	0	0	0	0	2	3	0	5
MME	0	0	0	0	8	2	0	10
PDO	0	0	1	1	22	9	13	46
SVU	0	0	4	0	1	0	0	5
TMI	27	8	101	3	0	1	1	141
QQU	0	0	0	0	5	1	0	6
TOTAL	27	8	108	4	51	36	15	

* CME and MAT does not sum to 16 and 10 across the AHY and HY tables as presented in Table 1 because 1 infected individual was not reliably aged for both species

Chapter 2

Variation in Passerine *Plasmodium* Prevalence is Associated with Exposure to Vectors

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Summary

The prevalence of avian malaria parasites varies greatly across host species, and this heterogeneity has been used to relate susceptibility to infectious diseases to host species traits. However, few empirical studies have directly associated avian *Plasmodium* prevalence and variation in exposure to malaria parasites as mediated by vector feeding patterns. Here, we estimate utilization of different passerine (Order: Passeriformes) host species by mosquitoes for blood meals. We show that passerine host species that are over-utilized by avian malaria vectors relative to their abundance are significantly more likely to have *Plasmodium* infections among an avian community. However, this effect was not consistent among individual *Plasmodium* species. Exposure to vector bites may therefore influence the relative number of total avian malaria infections of all parasite lineages among host species. Ecologists should be cautious about relating avian malaria susceptibility to host traits in the absence of information on 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.

keywords: avian malaria, *Plasmodium*, mosquito-feeding patterns, prevalence, disease exposure

Introduction

Host exposure and susceptibility to a pathogen are the fundamental determinants of infection. Infection dynamics driven by differential exposure and susceptibility among 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 ecology and evolution. For instance, host species prevalence have been linked to developmental rates (Ricklefs 1992, Tella *et al.* 1999), behavior and sociality (Nunn and Heymann 2005, Nunn *et al.* 2000, Fecchio *et al.* 2013), coloration (Hamilton and Zuk 1982, Scheuerlein and Ricklefs 2004), and habitat choice (Mendes *et al.* 2005), among other attributes.

Avian Haemosporida comprise a good system to explore the relationships between host species traits and infection rates, largely due to the substantial variation in prevalence between host species (Fallon *et al.* 2003, 2005, Latta *et al.* 2010), even within the same community (Ricklefs *et al.* 2005). Exposure to avian Haemosporida is mediated by biting dipteran vectors that transmit the parasites between hosts. However, the family of dipteran vector varies among the genera of parasite (Valkiunas 2005). For instance, *Haemoproteus* parasites are vectored by biting midges (Ceratopogonidae), while avian *Plasmodium* are transmitted by mosquitoes

(Culicidae). Some of these parasites are pathogenic to their hosts and infections are known to cause reduction in health and fitness among host individuals (Knowles et al. 2010, Martínez-de la Puente *et al.* 2010, Lachish *et al.* 2011).

Variation in haemosporidian prevalence among avian host species has been linked to plumage coloration and sexual selection (Hamilton and Zuk 1982, Scheuerlein and Ricklefs 2004), embryonic developmental periods (Ricklefs 1992, Tella *et al.* 1999), sociality (Fecchio *et al.* 2013), nesting characteristics (Garvin and Remsen 1997), habitat choice (Tella *et al.* 1999, Mendes *et al.* 2005), and body size (Scheuerlein and Ricklefs 2004). These relationships suggest that host traits either evolve as a result of high pathogen pressure or they predispose hosts to infection by modulating exposure or susceptibility to the parasite. For instance, Hamilton and Zuk (1982) suggested that bright plumage and elaborate secondary sexual characters evolved under parasite-mediated sexual selection, whereby costly plumage and displays demonstrated individual resistance among highly parasitized host species, thus driving a positive association between haemosporidian prevalence and plumage coloration among host species. In addition, Ricklefs 1992 proposed that prolonging the embryonic development periods of host species permitted the development of more competent immune systems, thus lowering infection rates of blood parasites including Haemosporida. While these proposed mechanisms for the association between prevalence and host species traits focus on differential susceptibility of host species, other explanations involving differential exposure to vectors may exist as well, as suggested by the original authors. For instance, brighter birds may attract more vectors

(Hamilton and Zuk 1982), although at least one study suggests that color alone may not drive this effect (Yezerinac and Weatherhead 1995). The young of birds with long developmental periods may spend more time in the relative protection of a nest (Ricklefs 1992), which may reduce vector encounter rates (Nunn *et al.* 2005). Thus, differential exposure to vectors complicates the interpretation of relationships between haemosporidian prevalence and host traits among different species.

Known avian *Plasmodium* vectors obtain blood meals heterogeneously with respect to available hosts species, often disproportionately to their relative abundance and availability in a community of potential hosts (Kilpatrick *et al.* 2006, Hamer *et al.* 2009). Thus, individuals of host that are over-utilized by vectors relative to their abundance may receive more vector bites than under-utilized species, thus providing more opportunities for infection with vector-borne pathogens. This heterogeneity underscores the risk in using host species traits as a proxy of susceptibility to avian Haemosporida when analyses do not control for variation in exposure to vectors among host species.

Ecological attributes of host species may influence exposure rates to suitable vectors, driving a correlation with haemosporidian prevalence. For instance, association with habitats that may harbor 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). Larger body size may be related to increased prevalence (Scheuerlein and Ricklefs 2004), potentially because larger

bodied birds attract more vectors. However, many studies attribute prevalence to variation in exposure to Haemosporida vectors without specifically linking particular host traits to heterogeneity in host-vector interactions. Fewer studies have directly related the distribution of Haemosporida with host-vector encounter rates (Hellgren *et al*. 2008, Gager *et al*. 2008, Medeiros *et al*. 2013). Gager *et al.* 2008 and Medeiros *et al.* 2013 suggested that associations with different vector species do not modulate the distribution of *Plasmodium* parasites among avian host species. Rather, host compatibility mechanisms involving differential susceptibility to various pathogen lineages likely play a larger role. However, both of these studies did not explicitly focus 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 explicitly relate patterns of host utilization by mosquito vectors to variation in avian *Plasmodium* prevalence among passerine (Order: Passeriformes) host species. We estimate the utilization of different passerine host species by two dominant avian *Plasmodium* vectors, *Culex pipiens* and *Culex restuans*. We use multinomial simulation to compare the number of observed *Culex* blood meals to an expected distribution across bird species based on host abundance. We show a positive association between over-utilization by both vector species and total *Plasmodium* prevalence among a community of suburban passerines in Chicago, IL, USA. However, this relationship breaks down when the prevalence of individual *Plasmodium* taxa is considered. Our results suggest a role of exposure to vectors in

explaining variation in total *Plasmodium* prevalence among bird species. However, the prevalence of individual lineages may be largely independent of vector feeding patterns, suggesting other forces modulate the prevalence of specific *Plasmodium* taxa across hosts.

Methods

Sampling and molecular analyses

Birds were captured in mist nets at 17 locations in suburban Chicago from May through October during 2006 and 2007. The age class and the date of each capture were recorded. Blood was obtained, centrifuged to separate cells from serum, and stored at -20°C for later analysis. DNA from the blood clot was extracted with a 5M ammonium acetate solution, and purified through a standard alcohol precipitation. We used PCR and DNA sequencing to diagnose haemosporidian infections (see Fallon *et al.* 2003, Ricklefs *et al.* 2005, Fecchio *et al*. 2013 for more details). Serum was also used to test for the presence of West Nile virus antibodies that indicate previous exposure to the pathogen (see Hamer *et al.* 2008). Bird surveys (point counts with distance sampling) were conducted across the same sites in 2006 to estimate host species abundances. Details about avian censuses are presented in Loss *et al.* 2009 and Hamer *et al.* 2009.

Blooded mosquitoes were sampled from the same sites during 2005-2008 with standard 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 source of blood meals from blood-fed mosquitoes was determined through established molecular protocols that included DNA extraction, selective amplification of vertebrate DNA through PCR, and sequencing of the amplicon (see Hamer *et al*. 2009).

Statistical Analyses

To analyze utilization by *Culex pipiens* and *Culex restuans* vectors across avian hosts, we used *rmultinom* from the stats package in program R to simulate the total number of avian blood meals collected from 2005-2008 (N=516 and 194 for *Culex pipiens* and *Culex restuans*, respectively). During the simulation, blood meals were assigned to host proportionately to their relative abundance in the community based on point-count surveys (see Loss *et al.* 2009). Simulations were repeated 100,000 times across the 43 bird species sampled during the point-count surveys, from which we extracted the resulting distributions and 95% confidence intervals for each host species.

We used the simulation to assess the relative rate of utilization by each vector by comparing the actual number of blood meals to this expected distribution generated by the simulation. First, we compared the actual number to the 95% confidence limits

from the expected distribution, and scored hosts as under-utilized or over-utilized following significant deviations ($\alpha = 0.05$). For each host species, we estimated a vector utilization index for each *Culex* species separately as the arcsine of the proportion of simulations that were less than or equal to the actual number of blood meals obtained.

We used generalized linear mixed modeling (GLMM) assuming a binomial error distribution in the R package "lme4" to investigate the effect of the vector utilization index on *Plasmodium* infection status under general recommendations of Bolker *et al.* 2009. Our analysis was limited to Passeriformes since the vast majority of species sampled here were passerines (~87%). We used three criteria to select passerine species for this analysis. (1) Species are widespread, summer residents of Chicago and occur in suburban habitat. This was determined by the presence of breeding adults or hatch-year birds in mist-netting records during June and July when birds are generally not migrating. The criterion increases the probability that included birds acquired their *Plasmodium* infections locally. (2) Species were sampled and screened 10 or more times to estimate *Plasmodium* infection prevalence. Bolker *et al.* 2009 recommends ≥10-20 replicates per random group level for a generalized linear mixed model. In addition, Jovani and Tella 2006 suggest that uncertainty in prevalence estimates decrease dramatically as the total sample reaches 10-20 individuals. Therefore, our cut-off is inclusive given these general recommendations. (3) Species make up over 0.1% of the total avian community. This stipulation was included to deal with issues associated with estimating vector utilization rates of

increasingly rare birds relative to the sample size of blood meals (see supplementary material for a more detailed discussion).

These criteria left us with 12 species in the analysis. Host species was a random effect and accounted for a large proportion of the variance in each model. We also included four covariates in addition to the numeric vector utilization index described above (age, year of sampling, month of sampling, West Nile virus serostatus) in the model since previous analyses demonstrated these were important predictors of *Plasmodium* infection, and they are unbalanced across species. The model estimates (β_{vector}) represent the change in the log-odds of infection for a unit change in the arcsine transformed utilization index. We assessed significance through a log-likelihood ratio test of nested models (i.e., one that included the vector utilization effect and another that did not). We performed the basic modeling approach detailed above for four separate analyses in which the dependent variable related to infection status was defined differently: 1) Total *Plasmodium*; 2) *Plasmodium cathemerium*, 3) *Plasmodium elongatum*, and 4) CHI07PL. These individual *Plasmodium* taxa were chosen because they were well sampled across the dataset. See Medeiros *et al.* 2013 for further characterization of these *Plasmodium* taxa.

Results

The simulations of *Culex* blood meals revealed that mosquitoes utilized some hosts disproportionately to their relative abundance as suggested by Hamer *et al.* 2009

(Table 1). Patterns of host utilization were similar between *Culex* species. The American robin (*Turdus migratorius*), northern cardinal (*Cardinalis cardinalis*), and house finch (*Carpodacus mexicanus*) were over-utilized by both *Culex pipiens* and *Culex restuans* based on their relative abundance in the community. House sparrows (*Passer domesticus*) and common grackles (*Quiscalus quiscula*) were under-utilized by both *Culex* mosquito vectors. In addition, European starling (*Sturnus vulgaris*), red-winged blackbird (*Agelaius phoeniceus*), and American goldfinch (*Carduelis tristis*) were under-utilized by *Culex pipiens*. The actual number of blood meals from other species fell within the expected 95% confidence limits based on their relative abundance in the community. See the supplementary table (Table S1) for a complete summary of the results from these simulations.

The utilization indices of both *Culex* mosquito vectors were positively associated with the prevalence of *Plasmodium* infection among the 12 well-sampled, suburban passerines analyzed ($p<0.05$, $p<0.01$ for *C. pipiens* and *C. restuans* respectively; Table 2, Figure 1). This suggests that passerine species that are over-utilized relative to their abundance have higher prevalence than species that are less utilized by vectors. However, the influence of vector utilization did not appear to strongly influence the probability of infection when individual *Plasmodium* taxa were considered separately (Table 2). Of six comparisons, only the *Culex restuans* utilization index was significantly related to *Plasmodium elongatum* infection probability ($p<0.05$, Table 2).

Discussion

Our results demonstrate a general association between exposure to mosquitoes and *Plasmodium* infection probabilities in an assemblage of avian hosts. Simple theoretical models of multi-pathogen vector-borne disease dynamics (such as the Ross-Macdonald model [Ross 1911, Macdonald 1957]) and other empirical investigations (Nevil *et al.* 1996, Charlwood *et al.* 1998, Martínez-de la Puente *et al.* 2013) suggest that this association 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, *Culex 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 *Culex pipiens* were less attracted to wild great tits (*Parus major*) with naturally acquired *Plasmodium* infections, calling the generality of this pattern into question.

While the prevalence of all *Plasmodium* infections together was positively related to vector utilization rates, prevalence of individual *Plasmodium* species appeared generally independent of exposure to vectors. This would be consistent with an upper limit to total *Plasmodium* prevalence set by mosquito biting rates, within which processes including parasite-parasite competition and host-parasite coevolution

determine more restricted host breadths of individual parasite species. These results largely corroborate a previous study within this system that suggests 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). Indeed, the positive relationship between host phylogenetic similarity and similarity in relationships with *Plasmodium* parasite taxa reported in that study highlight the role co-evolutionary forces may play in delimiting *Plasmodium* species host range (Apanius *et al.* 2000, Fallon *et al.* 2003). However, the lack of a relationship between *Plasmodium* taxa and vector feeding patterns may result as an artifact of a reduction in sample size. Increased sampling may be necessary to further explore this possibility.

Our results question the practice of relating avian malaria prevalence to species traits without controlling for differences in vector exposure. Mosquitoes feed heterogeneously on hosts, and our analysis suggests that this has consequences for 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 behaviors (Garvin and Remsen 1997), and body size (Scheuerlein and Ricklefs 2004) are often assumed to influence exposure to parasite vectors and the prevalence of vector-transmitted diseases. Additional studies relating these traits directly to vector utilization patterns would provide an improved understanding of the ecological drivers of variation in infection rates of vector-borne pathogens across host species.

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Tables and Figures

Table 1. Prevalence and *Culex* utilization rates.

Table 2. Summary of generalized linear mixed model results (GLMM).

Figure 1. Relationship between *Culex* sp. utilization indices and *Plasmodium*

prevalence across 12 well-sampled, suburban hosts. The utilization index represents the arcsine of the proportion of simulation values that were less than or equal to the actual number of vector blood meals derived from a host species. Regression lines result from weighted least-square regressions where weights are the square root of the host species sample size (number of blood samples screened for parasites), both of which were significant ($p<0.05$). Text in plots correspond to the AOU alpha-code for birds included in the analysis (AMRO = American robin (*Turdus migratorius*), HOFI = house finch (*Carpodacus mexicanus*), NOCA = northern cardinal (*Cardinalis cardinalis*), SOSP = song sparrow (*Melospiza melodia*), GRCA = gray catbird (*Dumetella carolinensis*), COGR = common grackle (*Quiscalus quiscula*), HOSP = house sparrow (*Passer domesticus*), RWBL = red-winged blackbird (*Agelaius phoeniceus*), CHSP = chipping sparrow (*Spizella passerina*), AMGO = American goldfinch (*Carduelis tristis*), BHCO = brown-headed cowbird (*Molothrus ater*), EUST = European starling (*Sturnus vulgaris*). Font size is proportional to number of birds sampled for parasites during the study.

Table 1.

	Culex pipiens Utilization Rates			*Culex restuans* Utilization Rates		
	Blood Meals	95% CL[1]	Utilization	Blood Meals	95% CL[1]	Utilization
American robin	255	92-128	Over-utilized	94	30-50	Over-utilized
northern cardinal	44	4-16	Over-utilized	20	0-8	Over-utilized
house finch	36	2-12	Over-utilized	8	0-6	Over-utilized
common grackle	2	25-45	Under-Utilized	2	7-21	Under-Utilized
house sparrow	76	201-245	Under-Utilized	32	70-97	Under-Utilized
Eurasian starling	12	22-44	Under-Utilized	12	6-19	Evenly-Utilized
red-winged blackbird	2	4-15	Under-Utilized	4	0-7	Evenly-Utilized *
American goldfinch	1	2-12	Under-Utilized	0	0-6	Evenly-Utilized *
chipping sparrow	2	2-11	Evenly-Utilized	0	0-5	Evenly-Utilized *
gray catbird	2	0-7	Evenly-Utilized *	2	0-4	Evenly-Utilized *
brown-headed cowbird	0	0-5	Evenly-Utilized *	2	0-3	Evenly-Utilized *
song sparrow	2	0-4	Evenly-Utilized *	1	0-2	Evenly-Utilized *

[1] CL is the confidence limits for the expected number of blood meals from the distributions generated by the simulations.

[2] * The CL include 0, and thus under-utilization cannot be identified given the sample size.

Table 2

	Culex pipiens GLMM results [1]			*Culex restuans* GLMM results [1]		
	β_{vector}	β_{vector} SE	P*	β_{vector}	β_{vector} SE	P*
Total *Plasmodium*	0.93	0.33	0.017	1.2	0.34	0.003
Plasmodium cathemerium	-0.11	0.47	0.822	-0.03	0.52	0.951
Plasmodium elongatum	0.90	0.65	0.176	1.52	0.66	0.026
CHI07PL	0.39	1.61	0.814	0.76	1.70	0.671

[1] Results are from a GLMM with month of capture, year of capture, age class, and WNV serostatus added as covariates. Species was added as a random effect.

* p-values based on log-likelihood ratio test of nested models

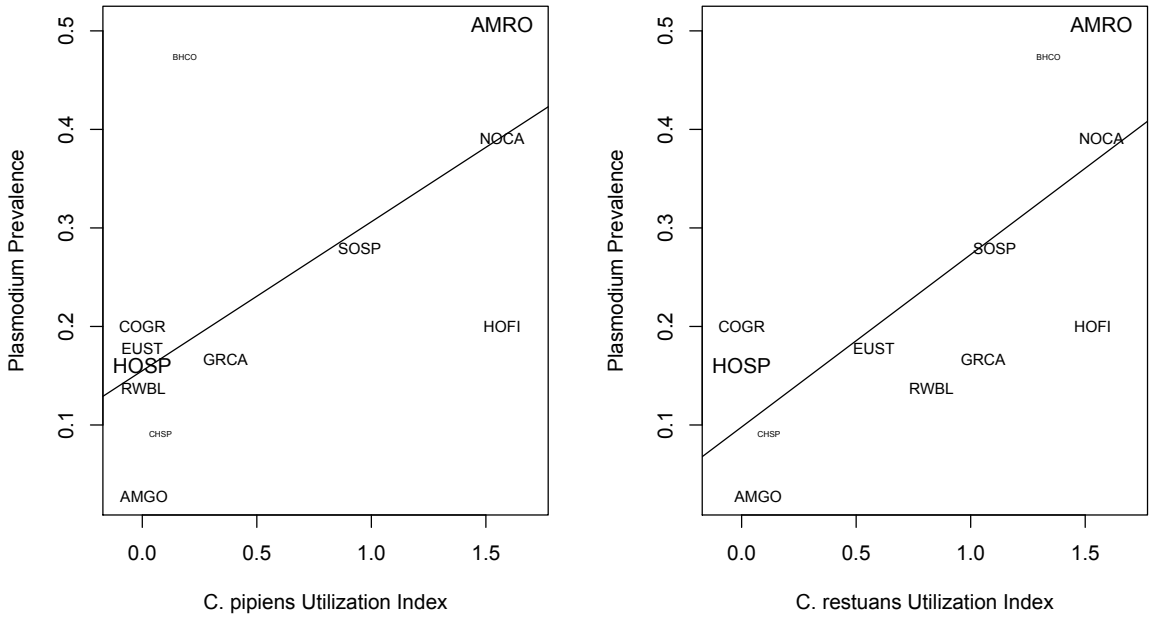
Plasmodium Prevalence
0.1
0.2
0.3
0.4
0.5
AMRO
BHCO
NOCA
SOSP
COGR
EUST
HOSP
GRCA
RWBL
HOFI
CHSP
AMGO
0.0
0.5
1.0
1.5
C. pipiens Utilization Index
Plasmodium Prevalence
0.1
0.2
0.3
0.4
0.5
AMRO
BHCO
NOCA
SOSP
COGR
EUST
HOSP
GRCA
RWBL
HOFI
CHSP
AMGO
0.0
0.5
1.0
1.5
C. restuans Utilization Index

Figure1.

Supplementary Information

Section 1. Full Blood Meal Simulation Results

Table S1. Summary of Blood Meal Simulation Results

Species Name	Proportion	C. pipiens Blood Meals	C. restuans Blood Meals	95% C. pipiens CL	95% C. restuans CL
House Sparrow	0.4323	76	32	201-245	70-97
American Robin	0.2124	255	94	92-128	30-50
Mourning Dove	0.0721	59	9	26-49	7-21
Common Grackle	0.0695	2	2	25-48	7-21
European Starling	0.0631	12	12	22-44	6-19
Monk Parakeet	0.0203	0	0	5-17	1-8
Northern Cardinal	0.0181	44	20	4-16	0-8
Red-winged Blackbird	0.0177	2	4	4-15	0-7
American Goldfinch	0.0134	1	0	2-12	0-6
Rock Dove	0.0129	1	0	2-12	0-6
House Finch	0.0121	36	8	2-12	0-6
Chipping Sparrow	0.0109	2	0	2-11	0-5
Cedar Waxwing	0.0065	3	0	0-7	0-4
Gray Catbird	0.0064	2	2	0-7	0-4
Blue Jay	0.0040	11	2	0-5	0-3
House Wren	0.0037	3	0	0-5	0-3
Brown-headed Cowbird	0.0033	0	2	0-5	0-3
Downy Woodpecker	0.0032	0	0	0-5	0-3
Song Sparrow	0.0029	2	1	0-4	0-2
Black-capped Chickadee	0.0024	2	0	0-4	0-2
Mallard	0.0016	0	1	0-3	0-2
American Crow	0.0013	0	0	0-3	0-2
Blue-gray Gnatcatcher	0.0012	0	0	0-3	0-1
Warbling Vireo	0.0009	0	0	0-2	0-1
Red-eyed Vireo	0.0009	0	0	0-2	0-1
Eastern Wood Pewee	0.0008	0	0	0-2	0-1
Veery	0.0007	1	1	0-2	0-1
White-breasted Nuthatch	0.0007	0	0	0-2	0-1
Great Crested Flycatcher	0.0006	0	0	0-2	0-1
Willow Flycatcher	0.0005	0	0	0-2	0-1
Eurasian Collared Dove	0.0005	0	0	0-2	0-1
Northern Flicker	0.0004	1	0	0-1	0-1
Eastern Kingbird	0.0004	0	0	0-1	0-1
Killdeer	0.0004	0	0	0-1	0-1
Common Yellowthroat	0.0003	0	0	0-1	0-1
Indigo Bunting	0.0003	0	0	0-1	0-1
Baltimore Oriole	0.0003	0	0	0-1	0-1
Eastern Bluebird	0.0002	0	1	0-1	0-1
Scarlet Tanager	0.0002	1	3	0-1	0-1
Eastern Towhee	0.0002	0	0	0-1	0-1
Hairy Woodpecker	0.0002	0	0	0-1	0-1
Yellow Warbler	<0.0001	0	0	0-0	0-0
Ring-necked Pheasant	<0.0001	0	0	0-0	0-0
Total	1.0000	516	194		

Section 2. Issues with Vector Utilization Indices

Numerous studies have estimated selection ratios of vectors on host species given their relative abundance and availability. This is often characterized as the ratio of the fraction of vector feeds on host *i* / (the density of host *i* / total density of hosts). This index (hereby referred to as the selection ratio) has successfully highlighted strong heterogeneity in the transmission dynamics of vector-borne pathogens (Kilpatrick *et al.* 2006, Hamer *et al.* 2009). However, the selection ratio has important shortcomings that are especially pertinent to our analysis presented here, primarily the uncertainty in characterizing vector utilization rates of rare hosts relative to more abundant ones. Here we highlight these shortcomings primarily with examples, and defend our species selection criteria presented in the methods concerning the minimum proportion of the community.

Given a finite sample size, the selection ratio (as well as other utilization indices) can inflate index values for rare species in the community. Consider a host species that makes up 0.0009 of the community (note this is just below our cut-off of 0.1% of the community). A single blood meal in a total sample of 194 (# of *Cx. restuans* blood meals identified here) would yield a selection ratio of (1/194)/.0009, or around 5.7. This would be rather high in the spectrum of values obtained for similar studies (Hamer *et al.* 2009). For comparison, American robins (which are arguably the most important host for mosquitoes) would yield a selection ratio of (94/194)/0.21, or 2.3. The odds of obtaining a blood meal by chance (ie. a multinomial simulation with a probability distribution based on relative abundance) for a host that makes up 0.0009

is $[1-(1-0.0009)^{194}]$, or 0.16. Therefore, the random sampling of 1 or more blood meal lies within the generally excepted α level of 0.05, yet it produces an inflated selection ratio.

Selection ratios may also be problematic because rare species have the potential for larger values than more common species since the maximum value is the inverse of the proportion. Thus, the maximum value for the most common host in our analysis (house sparrow) is 1/0.43, or 2.3 while the maximum value for the least dense host (song sparrow) is 1/0.003, or 333. Since this is a ratio of proportions, rare birds have a greater opportunity for higher selection ratios.

While our simulation approach mitigates these issues, it does not resolve them completely. For instance, consider the case of a rare bird like the Baltimore oriole (0.0003 of the community). Our simulations showed that 86% of samples of 516, and 94% of samples of 194 would yield 0 blood meals based on chance. Thus, our vector utilization index would be the arcsine of 0.86 and 0.94, for *Culex pipiens* and *Culex restuans* respectively. This yields relatively high estimates of vector utilization, especially given the fact that zero blood meals were obtained for Baltimore orioles from 2005-2008. This scenario is the primary reason for the minimum proportion of the community for inclusion in our analysis. We chose a cut-off such that a value of zero actual blood meals could only yield a proportion of simulations greater than or equal to the actual value obtained of around 0.5 or less, suggesting "even" utilization by vectors. Therefore, the cut-offs for both species were approximately $1-(0.5^{(1/516)})$

and $(1-0.5^{(1/194)})$, or 0.0013 and 0.0036 for *Culex pipiens* and *Culex restuans* respectively. We set the limit at 0.001, although functionally, this resulted in the scarcest bird (song sparrow) being 0.003 of the community.

Section 3. Selecting Host Species to Include in the General Linear Mixed Models

A summary of our designations for each passerine species across the three criteria is presented below in Table S2.

Table S2.

Species Name	Total Sampled	N>10	>0.1% of Community	Suburban
American Goldfinch	144	1	1	1
American Robin	425	1	1	1
Brown-headed Cowbird	19	1	1	1
Chipping Sparrow	11	1	1	1
Common Grackle	50	1	1	1
European Starling	62	1	1	1
Gray Catbird	150	1	1	1
House Finch	75	1	1	1
House Sparrow	517	1	1	1
Northern Cardinal	115	1	1	1
Red-winged Blackbird	51	1	1	1
Song Sparrow	69	1	1	1
American Crow	0	0	1	1
Baltimore Oriole	11	1	0	1
Black-capped Chickadee	5	0	1	1
Blue Jay	6	0	1	1
Cedar Waxwing	8	0	1	1
House Wren	3	0	1	1
Blue-gray Gnatcatcher	0	0	1	0
Eastern Kingbird	0	0	0	1
Eastern Towhee	2	0	0	1
Warbling Vireo	10	1	0	0
White-breasted Nuthatch	0	0	0	1
Willow Flycatcher	19	1	0	0
Common Yellowthroat	5	0	0	0
Eastern Bluebird	0	0	0	0
Eastern Wood Pewee	1	0	0	0
Great Crested Flycatcher	3	0	0	0
Indigo Bunting	6	0	0	0
Red-eyed Vireo	5	0	0	0
Scarlet Tanager	0	0	0	0
Veery	2	0	0	0
Yellow Warbler	6	0	0	0

[1] Birds within gray shaded cells were included in the vector utilization modeling of the main text.

Section 4. The Consequences of Species Selection Criteria

We assessed how our choice of criteria influenced our conclusions based on the analyses by further restricting and loosening the criteria of minimum sample size screened for parasites and proportion of the available community (Criteria 2-3 from the methods). Resident/suburban habitat use criteria remained unchanged. We identified passerine species that were < 1%, <0.1%, and < 0.01% of the community. In addition we identified passerines that had been sampled and screened for parasites ≥5, ≥10, and ≥20 times. We repeated all analyses on datasets of passerine hosts for all nine possible combinations of minimum values for each criteria (Table S3). We report the results for total *Plasmodium* and *Plasmodium elongatum* only. Patterns and significance did not change for *Plasmodium cathemerium* and CHI07PL when different criteria were used to select species for analyses.

Table S3. Summary of datasets

Criteria	Species Included
N≥5, ≥ 1.0% of Community	noca, cogr, rwbl, amro, amgo, eust, chsp, hosp, hofi
N≥5, ≥ 0.1% of Community	noca, grca, cogr, rwbl, amro, amgo, eust, sosp, chsp, bhco, hosp, hofi, bcch, blja, cedw
N≥5, ≥ 0.01% of Community	noca, grca, cogr, rwbl, amro, amgo, eust, sosp, chsp, bhco, hosp, hofi, bcch, blja, baor, cedw
N≥10, ≥ 1.0% of Community	noca, cogr, rwbl, amro, amgo, eust, chsp, hosp, hofi
N≥10, ≥ 0.1% of Community	**noca, grca, cogr, rwbl, amro, amgo, eust, sosp, chsp, bhco, hosp, hofi**
N≥10, ≥ 0.01% of Community	noca, grca, cogr, rwbl, amro, amgo, eust, sosp, chsp, bhco, hosp, hofi, baor
N≥20 ≥ 1.0% of Community	noca, cogr, rwbl, amro, amgo, eust, hosp, hofi
N≥20, ≥ 0.1% of Community	noca, grca, cogr, rwbl, amro, amgo, eust, sosp, hosp, hofi
N≥20, ≥ 0.01% of Community	noca, grca, cogr, rwbl, amro, amgo, eust, sosp, hosp, hofi

[1] Species abbreviations correspond to the AOU alpha-code for birds included in the analysis (amro = American robin (*Turdus migratorius*), hofi = house finch

(*Carpodacus mexicanus*), noca = northern cardinal (*Cardinalis cardinalis*), sosp = song sparrow (*Melospiza melodia*), grca = gray catbird (*Dumetella carolinensis*), cogr = common grackle (*Quiscalus quiscula*), hosp = house sparrow (*Passer domesticus*), rwbl = red-winged blackbird (*Agelaius phoeniceus*), chsp = chipping sparrow (*Spizella passerina*), amgo = American goldfinch (*Carduelis tristis*), bhco = brown-headed cowbird (*Molothrus ater*), eust = European starling (*Sturnus vulgaris*), cedw = cedar waxwing (*Bombycilla cedrorum*), blja = blue jay (*Cyanocitta cristata*), bcch = black-capped chickadee (*Poecile atricapillus*), baor = Baltimore oriole (*Icterus galbula*). Results from bolded criteria are presented in the main text.

Table S4. Summary of GLMM results with different selection criteria

a) total *Plasmodium*	N≥5		N≥10		N≥20	
	C.pipiens	*C.restuans*	*C.pipiens*	*C.restuans*	*C.pipiens*	*C.restuans*
≥ 1.0% of Community	β_{vector}= 1.1 p= 0.01	β_{vector}=1.2 p=0.01	β_{vector}= 1.1 p= 0.01	β_{vector}= 1.2 p= 0.01	β_{vector}= 1.0 p= 0.02	β_{vector}= 1.2 p= 0.02
≥ 0.1% of Community	β_{vector}= 0.7 p= 0.07	β_{vector} = 1.2 p< 0.01	β_{vector}= 0.9 p= 0.02	β_{vector}= 1.2 p< 0.01	β_{vector}= 1.0 p< 0.01	β_{vector}= 1.1 p= 0.01
≥ 0.01% of Community	β_{vector}= 0.6 p= 0.15	β_{vector}= 1.1 p= 0.01	β_{vector}= 0.8 p= 0.05	β_{vector}= 1.1 p= 0.01	β_{vector}= 1.0 p< 0.01	β_{vector}= 1.1 p= 0.01

b) *Plasmodium elongatum*	N≥5		N≥10		N≥20	
	C.pipiens	*C.restuans*	*C.pipiens*	*C.restuans*	*C.pipiens*	*C.restuans*
≥ 1.0% of Community	β_{vector}= 1.2 p= 0.08	β_{vector}= 1.3 p= 0.09	β_{vector}= 1.2 p= 0.08	β_{vector}= 1.3 p= 0.09	β_{vector}= 1.2 p= 0.09	β_{vector}= 1.3 p= 0.12
≥ 0.1% of Community	β_{vector}= 0.7 p=0.27	β_{vector}= 1.5 p= 0.02	β_{vector}= 0.9 p= 0.18	β_{vector}= 1.5 p= 0.03	β_{vector}= 1.1 p= 0.07	β_{vector}= 1.3 p= 0.06
≥ 0.01% of Community	β_{vector}= 0.7 p= 0.32	β_{vector}= 1.5 p= 0.03	β_{vector}= 0.8 p= 0.22	β_{vector}= 1.5 p= 0.04	β_{vector}= 1.1 p= 0.07	β_{vector}= 1.3 p= 0.06

Results of generalized linear mixed models for total *Plasmodium* (Table S4a) were fairly robust across different criteria. For *Culex restuans*, the slope of the vector utilization index and its level of significance ($p<0.05$) were consistent across the different inclusion criteria. However, including rare (≥ 0.01% of the community) and poorly sampled hosts ($5\leq N<10$), flattened the vector utilization index slope of *Culex pipiens,* making it insignificant. As indicated above, including rare host creates a large degree of uncertainty in the vector utilization index. In addition, prevalence estimates

for under sampled hosts are dubious as well (Bolker *et al.* 2009, Jovani and Tella 2006). Given the fact that these changes in the significance of the vector utilization slope were isolated to analysis that included poorly sampled, rare host with uncertain measurements, we argue that are conclusions based on the observed relationship between total *Plasmodium* infection probability and vector utilization presented in the main text remain valid.

In contrast to total *Plasmodium*, results of generalized linear mixed models for *Plasmodium elongatum* were fairly inconsistent across different criteria. The relationship between *Plasmodium elongatum* and the vector utilization of *Culex pipiens* was consistently insignificant across different inclusion criteria. However, the relationship between *Plasmodium elongatum* and the vector utilization of *Culex restuans* varied in its level of significance. Specifically, the relationship became insignificant when the analyses excluded rare, under sampled species (in contrast to the total *Plasmodium* effect). This was associated with removing brown-headed cowbirds, which have a high *C. restuans* utilization index and *Plasmodium elongatum* prevalence (Medeiros *et al.* 2013). We contend that the dependence of the effect on rare hosts makes this relationship more uncertain.

Chapter 3

An inverse association between West Nile virus serostatus and avian malaria infection status

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* Deceased

Summary

Infections of individual hosts by more than one pathogen may have important consequences for host fitness and pathogen transmission. Here, field investigations of haemosporidian and West Nile virus (WNV) infections in birds in suburban Chicago, Illinois, USA yielded an inverse association between WNV serostatus and *Plasmodium* infection status. This relationship occurred in adult birds but not in juveniles. There was no evidence for a relationship between *Haemoproteus* infection and WNV serostatus. We detected similar rates of *Plasmodium* infection among birds captured with active WNV infections and spatiotemporally paired WNV-naïve individuals of the same species, demonstrating that the two pathogens can co-infect hosts. Mechanisms explaining the negative association between WNV serostaus and *Plasmodium* infection status remain unclear but could be immunological or ecological.

Introduction

Numerous pathogens co-circulate within host populations, and various mechanisms influence the probability that these pathogens may cause concomitant or consecutive infections within a host individual (Telfer *et al.* 2010, Graham et al. 2007, Graham 2008, Enzewa *et al.* 2010). For instance, pathogens with similar ecological tolerances and vectors might be more likely to co-occur within a host (Atkinson *et al.* 2005), while differences may result in non-overlapping distributions across hosts over space and time. Infection can influence the susceptibility of a host to another pathogen (Telfer *et al.* 2010, Graham 2008), and this interaction can alter pathogen transmission dynamics at the population level (Enzewa *et al.* 2010). Negative associations between pathogens occur when an established pathogen lowers the infection success of another pathogen through immune-mediated mechanisms (Graham et al. 2007, Graham 2008) or resource competition (Graham 2008). Conversely, established parasites can increase the probability of infection of another pathogen through facilitation (Telfer *et al.* 2010, Ezenwa *et al.* 2010). Concomitant infections can elevate host morbidity and mortality rates (Graham *et al*. 2005), although this effect often depends on the particular pathogens involved (Knowles *et al.* 2011, Johnson and Hoverman 2012). Thus, pathogen-pathogen interactions can greatly influence the course of infection within the host and the distribution of pathogens among host individuals.

Avian Haemosporida of the genera *Plasmodium* and *Haemoproteus* are diverse and abundant parasites that infect a wide range of avian host species (Valkinuas 2005).

The two genera differ in their lifecycles and transmission dynamics (Atkinson and Van Riper 1991, Valkiunas 2005). Most notably, *Plasmodium* replicates asexually within erythrocytes leading to the rupture of blood cells, and is vectored by mosquitoes (Culicidae). In *Haemoproteus* infections, asexual reproduction is limited to the viscera and vascular endothelium, and the parasites are vectored by *Culicoides* biting midges (Ceratopogonidae). Haemosporidian parasites of both genera are often pathogenic (Knowles et al. 2010, Martínez-de la Puente *et al.* 2010, Lachish *et al.* 2011), but virulence varies with pathogen lineage and host species (Palinauskas *et al.* 2008, Lachish *et al.* 2011).

Avian Haemosporida are an instructive model system for disease ecology, including the consequences of concomitant infections. Mixed avian haemosporidian infections can be common within individuals in some host populations (van Rooyen *et al.* 2013). Both field- and laboratory-based studies have demonstrated that co-infection with different haemosporidian parasites can depress host fitness more than single infections (Marzal *et al.* 2008, Palinauskas *et al.* 2011). However, few studies have explored the interactions of avian Haemosporida with other pathogens (in contrast to mammalian malaria: Graham *et al.* 2005, Cox 2001, Knowles *et al.* 2011, however see Atkinson *et al*. 2005). This absence of knowledge is particularly concerning because birds are primary reservoirs for many zoonotic pathogens, including arthropod-borne encephalitis viruses whose course of infection may be influenced by concurrent protozoan infections (Cox 2001). Thus, understanding interactions between

Haemosporida and other pathogens could have important implications for disease surveillance.

Here, we explore patterns of association between avian Haemosporida and West Nile virus (WNV), an important zoonotic pathogen. WNV was introduced to North America in 1999 and spread across most of the continent within 5 years (Petersen and Hayes 2008, Kilpatrick *et al.* 2007). In 2012, one of the largest WNV epidemics to date occurred in North America (CDC). WNV is maintained in a transmission cycle generally between mosquitoes and birds, but is occasionally transmitted to other hosts, including humans (Kilpatrick *et al.* 2007). Symptoms of WNV infection in humans may be mild to severe, occasionally including neurologic impairment or death (Hayes *et al.* 2005). WNV infection also has severe fitness consequences for some avian host species (Yaremych *et al.* 2004), and its introduction coincided with population declines in some North American bird species (LaDeau *et al.* 2007). Associations between WNV and trypanosomes (van Dyken *et al.* 2006) and *Culex* flavivirus (Newman *et al.* 2011) have previously been documented in mosquitoes. However, interactions with Haemosporida within birds have received comparatively little attention.

The similar ecology of avian Haemosporida and WNV suggests that interactions between these pathogens might occur in North America. Both WNV and avian Haemosporida are common in the same host species. In North America, *Plasmodium* infections are common in American robins (*Turdus migratorius*),

northern cardinals (*Cardinalis cardinalis*), and house sparrows (*Passer domesticus*) (Medeiros *et al*. 2013). Individuals of these species also host WNV (Komar *et al.* 2003, 2005) and appear to be the vertebrate drivers of local WNV transmission dynamics at northern latitudes (Kilpatrick *et al.* 2006, Hamer *et al.* 2011). In addition, WNV and avian *Plasmodium* share the same vectors (*Culex pipiens* and *C. restuans*) in eastern North America (Valkiunas 2005, Medeiros *et al.* 2013, Kimura *et al.* 2010, Kilpatrick *et al.* 2005, Hamer *et al.* 2008), suggesting similar host encounter rates between the pathogens. In this study, we explore the potential interaction between WNV and Haemosporida near Chicago, IL, USA, and show that WNV seropositive birds generally have a lower probability of haemosporidian infection.

Methods

Sampling and Pathogen Testing

The study was conducted at 17 sites in suburban Chicago, IL, USA (Loss *et al.* 2009). Birds were captured in mist-nets from May-October during 2006-2007. A blood sample was taken from the jugular vein and centrifuged to separate serum from blood cells. Packed blood cells were preserved in Longmire's lysis buffer. Samples were digested with Proteinase K overnight at 60° C. DNA was extracted via protein precipitation with 5M ammonium acetate, and purified with a standard alcohol precipitation. DNA samples were screened for haemosporidian parasites by polymerase chain reaction (PCR) targeting a segment of the mitochondrial 16S rRNA

gene (Fallon *et al* 2003). Samples that tested positive by this method were then subjected to a nested PCR that targeted a 552-base pair fragment of the haemosporidian cytochrome *b* gene (Ricklefs *et al.* 2005, nested reaction summarized in Fecchio *et al*. 2013). We generally obtained sequences from ~85% of samples that screened positive for a haemosporidian infection with the 16S rRNA primers.

Avian haemosporidian taxonomy is unresolved at the species level, and currently relies on cytochrome *b* sequences to identify parasite taxa (Bensch *et al.* 2004, Martinsen *et al*. 2006, 2007). We therefore separated evolutionary lineages of *Plasmodium* and *Haemoproteus* following Ricklefs *et al.* (2005). Generally, haemosporidian taxa were categorized as sets of closely related (<1% sequence divergence) monophyletic parasite cytochrome *b* haplotypes recovered from the same set of host species. Two lineages presented here were identical to previously named morphospecies (*Plasmodium cathemerium* and *Plasmodium elongatum,* Genbank accession no. AY377128 and AY733088, respectively).

Avian serum was used to test for the presence of WNV antibodies using inhibition ELISA (Hamer *et al*. 2008), and to screen individuals for circulating WNV with a quantitative reverse transcriptase-PCR (Hamer *et al.* 2008). Among birds screened for malaria, ~7% were seropositive for WNV antibodies. From 2005-2011, 5728 birds were screened for WNV. Only 27 (0.5%) were positive for the virus by this method (Hamer *et al.* 2013). To compare concomitant infection rates of WNV and Haemosporida, blood samples from 26 of these birds were screened for

haemosporidian infections. In addition, we screened 26 spatiotemporally paired WNV-negative samples of the same species and age. Each WNV positive and negative pair was sampled from the same site, generally on the same day.

Haemosporidian Phylogeny

We used cytochrome *b* sequences (512bp) to reconstruct phylogenetic relationships among haemosporidian parasites by maximum likelihood, invoking the GTR+γ model of evolution implemented in the MEGA5 (Tamura *et al.* 2011). The tree was mid-point rooted. The resulting tree (Supplementary Material, Figure S1) was used primarily to assign lineages to genera.

Statistical Analyses

We used a series of generalized linear mixed models (GLMM) with a binomial error distribution to test for an association between WNV serostatus and Haemosporida infection status. All GLMMs were performed in the lme4 package in R. Our data were heterogeneous and unbalanced with respect to other variables that potentially influence haemosporidian infection across WNV-seropositive and naïve individuals. Therefore, we included species as a random factor in all models tested. Moreover, year of sampling (two levels: 2006 and 2007), month of sampling (four levels: May/June, July, August, September/October), age class at sampling (two levels: hatch-year juvenile [HY] or after hatch-year adult [AHY]), WNV serostatus (presence or

absence of WNV antibodies), and an age*WNV serostatus interaction were included as covariates in a full model.

We used AICc multimodel inference to select among a set of candidate models (Supplementary Material-Section 2) that included all combinations of the five fixed effect variables. We estimated the natural average of the estimate and associated unconditional 95% confidence intervals (Burnham and Anderson 2002) with the R package "AICcmodavg". WNV serostatus model estimates (β_{WNV}) represent the change in the log-odds of Haemosporida infection for WNV seropositive relative to WNV seronegative individuals. Within the text, values of β_{WNV} are presented as the natural average of the model coefficients ± 1.96*unconditional (model-averaged) standard error (SE). When calculating the model-averaged β_{WNV}, we excluded models with the age*WNV interaction term. We performed the basic modeling approach detailed above for five separate analyses in which the dependent variable of infection status was defined differently: 1) total Haemosporidia; 2) total *Plasmodium*, 3) total *Haemoproteus*, 4) *Plasmodium cathemerium*, and 5) *Plasmodium elongatum*. We performed the basic modeling approach detailed above for data on 1714 individuals of 13 well-sampled species (N>10) that had both haemosporidian infections and WNV seropositive individuals. We excluded records of other species that did not fit the criteria above, or those with missing data (ie. unknown age, WNV serological status, etc). All species along with their sample sizes across age class, *Plasmodium* prevalence, *Haemoproteus* prevalence, and WNV seroprevalence are listed in Table S1.

Results

WNV Serostatus and Haemosporida Infection

The best-fit model explaining total Haemosporida infection status included month of capture, year of capture, age, WNV serostatus, and age*WNV serostatus interaction (w_i =0.97), and was differentiated from other models (Table 1). Thus, we split the dataset across age class to investigate the interaction. For adults, WNV serostatus was an important predictor of Haemosporida infection (Table 2). The best-fit model included year and WNV serostatus (w_i=0.69) and was differentiated from a model that only included year as a fixed effect (ΔAICc=5.5, w_i=0.04). The model-averaged WNV serostatus effect (β_{WNV}=-0.78±0.59) indicated that the presence of WNV antibodies reduced the odds of a concomitant haemosporidian infection by a factor of 2.2 for adult birds. Among juvenile birds, the best-fit model included month and year of capture (w_i=0.73), and was differentiated from a model that contained month, year, and WNV serostatus (ΔAICc=2.0, w_i=0.27). The confidence limits of the WNV serostatus effect (β_{WNV}=-0.06±0.94) for juvenile hosts included zero, providing low support for an association between WNV serostatus and Haemosporida infection among hatch-year birds.

The best-fit model explaining total *Plasmodium* infection status included month of capture, year of capture, age, WNV serostatus, and age*WNV serostatus

interaction (w_i =0.99), and was well differentiated from other models (Table 1). Splitting the dataset across age classes, the best-fit model for adult birds included year and WNV serostatus (w_i =0.64, Table 2), but was indistinguishable from a model that included month, year, and WNV serostatus (ΔAICc=1.2, w_i=0.35). The model-averaged WNV serostatus effect (β_{WNV}=-1.34±0.84) indicated that the presence of WNV antibodies reduced the odds of a concomitant *Plasmodium* infection by a factor of 3.8 for adult birds (Figure 1). For juvenile birds, the best-fit model included month and year of capture (w_i=0.72, Table 2), but was indistinguishable from a model that included those fixed effects and WNV serostatus (ΔAICc=1.9, w_i=0.28). The confidence limits of the WNV serostatus effect (β_{WNV}= -0.18±0.94) for juvenile hosts included zero, providing low support for an association between WNV serostatus and *Plasmodium* infection among hatch-year birds.

We found no support for an association between WNV serostatus and *Haemoproteus* infection (Table 1). The best-fit model included month (w_i=0.32), however, three other models had ΔAICc < 3 (Table 1). The confidence limits of the WNV serostatus effect on *Haemoproteus* included zero (β_{WNV}= 0.08±0.67).

Results with two well-sampled *Plasmodium* lineages were broadly similar to those obtained with total *Plasmodium*. The best fit model for *Plasmodium cathemerium* included the fixed effects of month, year, age, and WNV serostatus (w_i=0.59, Table 1), but was indistinguishable from a model that included those fixed effects and the age*WNV serostatus interaction (ΔAICc=1.8, w_i=0.24). However, the

best-fit model was differentiated from a model that included only month, year, and age (ΔAICc=4.0, w_i=0.08). The model-averaged WNV serostatus effect (β_{WNV}=-1.22±1.10) indicated that the presence of WNV antibodies reduced the odds of a concomitant *P. cathemerium* infection by a factor of 3.4 for adult birds. The best-fit model for *Plasmodium elongatum* included the fixed effects of year, age, and WNV serostatus (w_i=0.40, Table 1), but was indistinguishable from a model that included only year and age (ΔAICc=1.4 w_i=0.20). The model-averaged WNV serostatus estimate (β_{WNV}=-0.89±1.0) indicated that the presence of WNV antibodies reduced the odds of a concomitant *P. elongatum* infection by a factor of 2.4, although the confidence limits of the estimate included zero.

Full AICc tables for all analyses are shown in the supplementary online material, Tables S2-11.

Analysis of WNV-positive birds

Nine birds of 26 that were positive for WNV had Haemosporida infections, eight of which were *Plasmodium*. A similar number (8/26) of spatiotemporally paired individuals of the same species that did not test positive for WNV had haemosporidian infections (p=1.0, fisher exact test). All of these were *Plasmodium* infections (Table 3). WNV-infected birds appear to be infected with a diversity of Haemosporida at a comparable rate relative to birds without WNV infections (Table 3).

Discussion

Haemosporida are common parasites of suburban birds in North America, yet little is known about their potential interactions with WNV. Our data demonstrate a negative association between the presence of WNV antibodies and avian Haemosporida infection among urban birds of Chicago. However, this negative association was context-dependent, varying with respect to haemosporidian taxonomy and host age. The presence of WNV antibodies was associated with a lower probability of infection with avian *Plasmodium* taxa, but not *Haemoproteus*. Moreover, the inverse association between WNV serostatus and *Plasmodium* infection status was present mainly in adult birds. In contrast to the WNV serostatus effect, birds that had an active WNV infection were equally likely to have a *Plasmodium* infection as birds that did not have a WNV infection. These data suggest that WNV and *Plasmodium* parasites do co-occur and potentially interact within hosts.

Several non-mutually exclusive mechanisms might account for the negative association between the presence of WNV antibodies and the probability of infection with avian *Plasmodium*. First, confounding ecological factors may result in patterns consistent with real interactions between pathogens, even though these interactions do not actually occur within hosts (Hellard *et al*. 2012). Shared hosts and vectors predict a passive positive association between the pathogens. However, differing temporal patterns of WNV-seropositive and *Plasmodium*-infected hosts (perhaps related to environmental variables like temperature) might produce an apparent negative

association between the pathogens. *Plasmodium* infections generally appear earlier within a transmission season than WNV antibodies among adult birds in Chicago, IL (see SOM-Section 3). However, analyses focused on a period when WNV seropositive and *Plasmodium* infected hosts overlap temporally showed a negative association between the presence of WNV antibodies and *Plasmodium* (SOM-Section 3). This suggests that asynchronous infection dynamics do not solely drive the inverse relationship between WNV serostatus and *Plasmodium* infection status.

Second, WNV and *Plasmodium* may compete directly within a host. Direct competition for host nutrients or cell types could reduce co-occurrence between the pathogens within a host. WNV is known to cause anemia in some birds (Joyner *et al.* 2006), and thus may reduce the amount red blood cells available to *Plasmodium* parasites. The availability of red blood cells may influence the invasion success of *Plasmodium* parasites, parasitemia, or the persistence of parasites within the bloodstream. For instance, anemia-inducing helminths lower the parasitemia of microparasites that require red blood cells in rodents (Graham 2008). However, direct competition would be expected to occur in both juvenile and adult hosts similarly. The apparent restriction of the WNV serostatus effect to adult hosts may suggest this mechanism is less likely.

Third, WNV and *Plasmodium* parasites may interact indirectly, mediated through host physiology. For instance, a pathogen may "prime" a host's immune system to respond to a secondary pathogen and thus influence the potential for co-

infection (Graham *et al.* 2007). While direct crossover immunity would not be expected between WNV and *Plasmodium* given the biological differences between the pathogens, suppression of one infection by the other has been reported for concomitant infections of *Plasmodium* and other viruses (Cox *et al.* 2001). These effects may be mediated by the Th1 and Th2 polarization of mammalian (Mosmann *et al.* 1986) and avian immune systems (Schwarz *et al.* 2011). While immune responses to both pathogens are varied, viruses and intacellular microparasites like *Plasmodium* typically activate a Th1 response associated with cell-mediated immunity. Since both WNV and *Plasmodium* elicit the same general cytokine response, the immune response toward one pathogen may also counteract infections by the other (Graham *et al*. 2007).

Alternatively, co-infection with WNV and *Plasmodium* may reduce host survival, producing an apparent negative association between these pathogens among hosts. Both *Plasmodium* and WNV can produce broad pathological changes in infected avian hosts and fitness consequences for hosts have been documented for each pathogen independently (Valkiunas 2005, Cellier-Holzem *et al.* 2010, Palinauskas *et al.* 2008, Steele *et al.* 2000). Co-infections may induce an additive effect on mortality probabilities, either by disrupting important physiological processes, or making death by other extrinsic factors (ie. predation) more likely (Moller & Nielsen 2007). Given broad differences in the physiology and causes of mortality between juvenile and adult birds, mechanisms mediated by host physiology could produce different outcomes of pathogen association across age class.

Identifying the mechanisms that drive an inverse association between WNV serostatus and *Plasmodium* infection status may provide integrated perspectives on host health and demography because alternate mechanisms may impact host survival and disease transmission dynamics differently (Graham et al. 2007). For instance, if co-infections elevate the risk of host mortality, *Plasmodium* may have played a role in declines of North American bird populations following the introduction of WNV (LaDeau *et al.* 2007). Interactions with *Plasmodium* could also impact WNV transmission. If a *Plasmodium* infection primes the immune system against WNV, *Plasmodium* transmission might lower the average host competence for WNV and reduce the potential for WNV transmission. Alternatively, existing *Plasmodium* infections could prolong or intensify a WNV infection, increasing the transmission potential or force of infection exerted by the host. Additionally, if co-infection is associated with increased host mortality, WNV transmission could be impacted by a reduction in recovered hosts that act as sinks in the transmission cycle. Ultimately, controlled experimental infection studies are necessary to understand WNV-*Plasmodium* interactions, and test mechanisms that may produce the inverse association between WNV serostatus and *Plasmodium* infection status among avian hosts. Such studies could shed more light on the implications of potential interactions between these pathogens among wild birds.

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Tables and Figures

Figure 1. *Plasmodium* prevalence of WNV-seropositive and WNV-seronegative adult birds across host species. The numbers above bars represent sample sizes. European starlings (*Sturnus vulgaris*), mourning doves (*Zenaida macroura*), and brown-headed cowbirds (*Molothrus ater*) were not included because of a total lack of *Plasmodium* or WNV infections among adult birds. Labels correspond to 4-letter abbreviated AOU alpha codes. AMRO = American robin (*Turdus migratorius*), HOFI = house finch (*Carpodacus mexicanus*), NOCA = northern cardinal (*Cardinalis cardinalis*), SOSP = song sparrow (*Melospiza melodia*), GRCA = gray catbird (*Dumetella carolinensis*), COGR = common grackle (*Quiscalus quiscula*), HOSP = house sparrow (*Passer*

domesticus), RWBL = red-winged blackbird (*Agelaius phoeniceus*), CHSP = chipping sparrow (*Spizella passerina*), AMGO = American goldfinch (*Carduelis tristis*).

Table 1. A summary AICc table for generalized linear mixed models that considered both host ages simultaneously and an age*WNV serostatus interaction effect. Models with ΔAICc < 3.0 are shown. However, when only one model had a ΔAICc < 3.0, the next best model was listed for comparison. Abbreviations are as follows: mon= month of capture, yr = year of capture, age = age class, wnv = WNV serostatus (seropositive, seronegative). Species was a random effect in all models tested.

Table 2. A summary AICc table for generalized linear mixed models performed on adults and juveniles separately. Only models with ΔAICc < 3.0 are shown. Abbreviations are as follows: mon= month of capture, yr = year of capture, wnv = WNV serostatus (seropositive, seronegative). Species was a random effect in all models tested.

Table 3. Number of Haemosporida infections and total host individuals sampled among birds with active WNV infections and spatiotemporally paired individuals of the same age and host species that were naïve to WNV. Haemosporida lineages recovered from each host are listed.

Table 1.

	K	AICc	ΔAICc	w_iAICc
a) Total Haemosporidia				
mon+yr+age+**wnv**+**wnv*age**	9	2056.5	0.0	0.97
mon+yr+age+**wnv**	8	2064.0	7.5	0.02
b) Total *Plasmodium*				
mon+yr+age+**wnv**+**wnv*age**	9	1704.7	0.0	0.99
mon+yr+age+**wnv**	8	1713.9	9.1	0.01
c) Total *Haemoproteus*				
mon	5	742.3	0.0	0.32
mon+yr	6	744.1	1.8	0.13
mon+age	6	744.2	1.9	0.12
mon+**wnv**	6	744.3	2.0	0.12
d) *P. cathemerium*				
mon+yr+age+**wnv**	8	823.4	0.0	0.59
mon+yr+age+**wnv**+**wnv*age**	9	825.2	1.8	0.24
e) *P. elongatum*				
yr+age+**wnv**	5	586.23	0.0	0.40
yr+age+**wnv**+**wnv*age**	6	587.0	0.8	0.27
yr+age	4	587.6	1.4	0.20

Table 2.

	K	AICc	ΔAICc	w_iAICc
a)Total Haemosporidia-adults				
yr+**wnv**	4	1138.9	0.0	0.69
mon+yr+**wnv**	7	1140.9	2.1	0.24
b)Total Haemosporidia-juveniles				
mon+yr	6	862.7	0.0	0.73
mon+yr+**wnv**	7	864.7	2.0	0.27
c) Total *Plasmodium*-adults				
yr+**wnv**	4	871.4	0.0	0.64
mon+yr+**wnv**	7	872.6	1.2	0.35
d) Total *Plasmodium*-juveniles				
mon+yr	6	785.5	0.0	0.72
mon+yr+**wnv**	7	787.4	1.9	0.28

a) West Nile virus positive

	Haemosporida Infections	*Plasmodium* Infections	Total Sampled	Haemosporidian Lineages
American robin[1]	2	1	2	CHI04PL, CHI02PL, CHI19PA
gray catbird	1	0	1	CHI01PA
house finch	0	0	3	
house sparrow	4	4	15	*P. cathemerium*, CHI05PL, *P. elongatum*
house wren	0	0	1	
northern cardinal	2	2	2	*P. cathemerium*, *P. elongatum*
red-winged blackbird	0	0	1	
northern flicker	0	0	1	
Total	9	7	26	

b) West Nile virus negative

American robin[1]	1	1	2	CHI04PL[1], *P. elongatum*
gray catbird	0	0	1	
house finch	1	1	3	*P.cathemerium*
house sparrow[2]	4	2	15	*P.cathemerium*, *P. elongatum*
house wren	0	0	1	
northern cardinal	1	1	2	*P. elongatum*
red-winged blackbird	1	1	1	*P.cathemerium*
northern flicker	0	0	1	
Total	8	6	26	

Table 3.

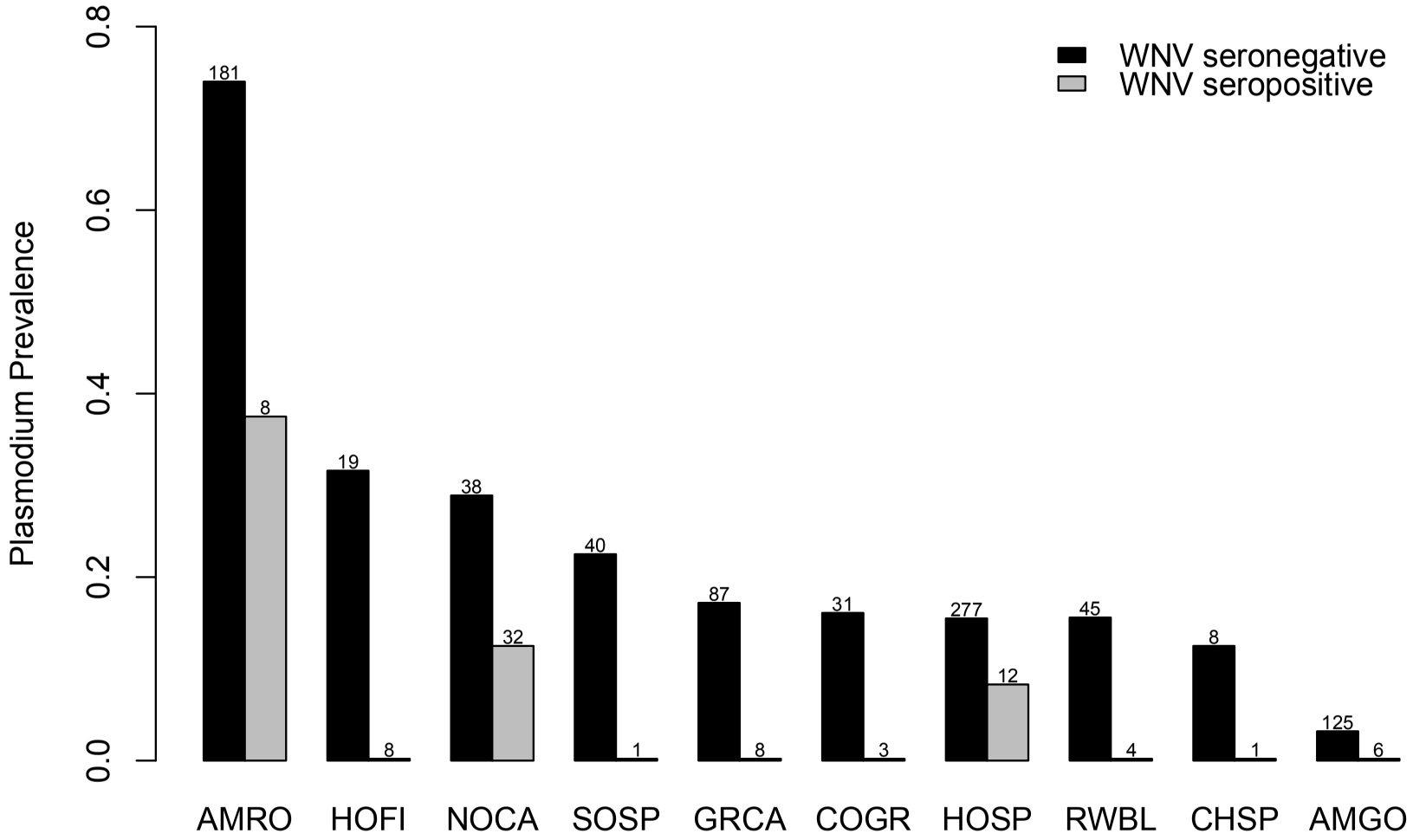
Plasmodium Prevalence
0.0
0.2
0.4
0.6
0.8
WNV seronegative
WNV seropositive
181
8
19
8
38
32
40
1
87
8
31
3
277
12
45
4
8
1
125
6
AMRO
HOFI
NOCA
SOSP
GRCA
COGR
HOSP
RWBL
CHSP
AMGO

Figure 1.

Supplementary Information

Section I. Supplementary Tables-Table S1

Species	N	N (AHY/HY)[1]		*Plasmodium* Prevalence	*Haemoproteus* Prevalence	WNV Seroprevalence
American goldfinch	144	131	13	0.03	0.15	0.04
American robin	425	189	236	0.51	0.05	0.03
brown-headed cowbird	19	14	5	0.47	0.05	0.05
chipping sparrow	11	9	2	0.09	.36	0.09
common grackle	50	34	16	0.20	0.32	0.06
Eurasian starling	62	29	33	0.18	0	0.03
gray catbird	150	95	55	0.17	0.16	0.07
house finch	75	27	48	0.20	0	0.16
house sparrow	517	289	228	0.17	0	0.03
mourning dove	26	15	11	0	0.62	0.35
northern cardinal	115	70	45	0.39	0.17	0.35
red-winged blackbird	51	49	2	0.14	0.16	0.10
song sparrow	69	41	28	0.28	0.01	0.01
Community Totals	1714	992	722	0.26	0.08	0.07

[1]After-hatch-year (AHY) and hatch-year (HY) birds

Section II. Model Selection

Table S2. AICc table for models predicting Haemosporida infection across all age classes.

Models	K	AICc	Δ AICc	AICc *w*
mon+yr+age+wnv+wnv:age+(1|Species)	9	2056.5	0.0	0.97
mon+yr+age+wnv+(1|Species)	8	2064.0	7.5	0.02
yr+age+wnv+wnv:age+(1|Species)	6	2069.8	13.3	0.00
mon+yr+age+(1|Species)	7	2071.5	15.0	0.00
mon+yr+wnv+(1|Species)	7	2072.9	16.3	0.00
yr+age+wnv+(1|Species)	5	2077.6	21.1	0.00
mon+yr+(1|Species)	6	2077.8	21.3	0.00
yr+wnv+(1|Species)	4	2081.8	25.3	0.00
yr+age+(1|Species)	4	2083.8	27.3	0.00
yr+(1|Species)	3	2086.5	30.0	0.00
mon+age+wnv+wnv:age+(1|Species)	8	2088.9	32.4	0.00
mon+age+wnv+(1|Species)	7	2098.2	41.7	0.00
age+wnv+wnv:age+(1|Species)	5	2100.2	43.7	0.00
mon+wnv+(1|Species)	6	2109.3	52.8	0.00
mon+age+(1|Species)	6	2109.4	52.9	0.00
age+wnv+(1|Species)	4	2109.9	53.4	0.00
wnv+(1|Species)	3	2115.9	59.4	0.00
mon+(1|Species)	5	2117.2	60.7	0.00
age+(1|Species)	3	2119.6	63.1	0.00
1+(1|Species)	2	2123.7	67.2	0.00

Abbreviations: mon= month of capture (4 levels: May/June, July, August, September/October), yr = year of capture (2 levels:2006, 2007), age (2 levels: hatch year juvenile, after hatch-year adult), wnv = WNV serostatus (seropositive, seronegative). Species (13 groups) was a random effect in all models tested. K = # of model parameters, AICc *w*= AICc weight

Table S3. AICc table for models predicting *Plasmodium* infection across all age classes.

Models	K	AICc	Δ AICc	AICc *w*
mon+yr+age+wnv+wnv:age+(1|Species)	9	1704.7	0.0	0.99
mon+yr+age+wnv+(1|Species)	8	1713.9	9.1	0.01
mon+yr+wnv+(1|Species)	7	1721.9	17.2	0.00
mon+yr+age+(1|Species)	7	1725.1	20.4	0.00
yr+age+wnv+wnv:age+(1|Species)	6	1727.3	22.5	0.00
mon+yr+(1|Species)	6	1730.4	25.7	0.00
mon+age+wnv+wnv:age+(1|Species)	8	1731.1	26.4	0.00
yr+age+wnv+(1|Species)	5	1737.1	32.4	0.00
yr+wnv+(1|Species)	4	1739.3	34.6	0.00
mon+age+wnv+(1|Species)	7	1742.2	37.5	0.00
yr+age+(1|Species)	4	1745.4	40.6	0.00
yr+(1|Species)	3	1746.4	41.6	0.00
age+wnv+wnv:age+(1|Species)	5	1748.9	44.2	0.00
mon+wnv+(1|Species)	6	1752.4	47.6	0.00
mon+age+(1|Species)	6	1757.7	53.0	0.00
age+wnv+(1|Species)	4	1760.6	55.9	0.00
mon+(1|Species)	5	1764.4	59.6	0.00
wnv+(1|Species)	3	1764.5	59.7	0.00
age+(1|Species)	3	1772.7	68.0	0.00
1+(1|Species)	2	1774.8	70.1	0.00

Abbreviations: mon= month of capture (4 levels: May/June, July, August, September/October), yr = year of capture (2 levels:2006, 2007), age (2 levels: hatch year juvenile, after hatch-year adult), wnv = WNV serostatus (seropositive, seronegative). Species (13 groups) was a random effect in all models tested. K = # of model parameters, AICc *w*= AICc weight

Table S4. AICc table for models predicting *Haemoproteus* infection across all age classes.

Models	K	AICc	Δ AICc	AICc *w*
mon+(1|Species)	5	742.3	0.0	0.32
mon+yr+(1|Species)	6	744.1	1.8	0.13
mon+age+(1|Species)	6	744.2	1.9	0.12
mon+wnv+(1|Species)	6	744.3	2.0	0.12
mon+yr+age+(1|Species)	7	746.1	3.8	0.05
mon+yr+wnv+(1|Species)	7	746.1	3.8	0.05
mon+age+wnv+(1|Species)	7	746.2	3.9	0.05
1+(1|Species)	2	746.7	4.4	0.04
mon+age+wnv+wnv:age+(1|Species)	8	747.6	5.3	0.02
mon+yr+age+wnv+(1|Species)	8	748.0	5.6	0.02
age+(1|Species)	3	748.2	5.9	0.02
yr+(1|Species)	3	748.5	6.1	0.01
wnv+(1|Species)	3	748.7	6.4	0.01
mon+yr+age+wnv+wnv:age+(1|Species)	9	749.4	7.1	0.01
yr+age+(1|Species)	4	749.9	7.6	0.01
age+wnv+(1|Species)	4	750.2	7.9	0.01
yr+wnv+(1|Species)	4	750.5	8.2	0.01
age+wnv+wnv:age+(1|Species)	5	751.6	9.2	0.00
yr+age+wnv+(1|Species)	5	751.9	9.6	0.00
yr+age+wnv+wnv:age+(1|Species)	6	753.3	11.0	0.00

Abbreviations: mon= month of capture (4 levels: May/June, July, August, September/October), yr = year of capture (2 levels:2006, 2007), age (2 levels: hatch year juvenile, after hatch-year adult), wnv = WNV serostatus (seropositive, seronegative). Species (13 groups) was a random effect in all models tested. K = # of model parameters, AICc *w*= AICc weight

Table S5. AICc table for models predicting *Plasmodium cathemerium* infection across all age classes.

Models	K	AICc	Δ AICc	AICc *w*
mon+yr+age+wnv+(1|Species)	8	823.4	0.0	0.59
mon+yr+age+wnv+wnv:age+(1|Species)	9	825.2	1.8	0.24
mon+yr+age+(1|Species)	7	827.4	4.0	0.08
yr+age+wnv+(1|Species)	5	827.8	4.4	0.07
yr+age+wnv+wnv:age+(1|Species)	6	829.5	6.2	0.03
yr+age+(1|Species)	4	831.9	8.5	0.01
mon+yr+wnv+(1|Species)	7	836.0	12.7	0.00
mon+age+wnv+(1|Species)	7	841.3	18.0	0.00
age+wnv+(1|Species)	4	842.1	18.7	0.00
yr+wnv+(1|Species)	4	842.7	19.3	0.00
mon+age+wnv+wnv:age+(1|Species)	8	842.9	19.5	0.00
mon+yr+(1|Species)	6	843.1	19.8	0.00
age+wnv+wnv:age+(1|Species)	5	843.6	20.3	0.00
mon+age+(1|Species)	6	849.1	25.8	0.00
yr+(1|Species)	3	849.2	25.8	0.00
age+(1|Species)	3	849.5	26.2	0.00
mon+wnv+(1|Species)	6	850.9	27.6	0.00
wnv+(1|Species)	3	854.0	30.6	0.00
mon+(1|Species)	5	862.6	39.2	0.00
1+(1|Species)	2	864.5	41.2	0.00

Abbreviations: mon= month of capture (4 levels: May/June, July, August, September/October), yr = year of capture (2 levels:2006, 2007), age (2 levels: hatch year juvenile, after hatch-year adult), wnv = WNV serostatus (seropositive, seronegative). Species (13 groups) was a random effect in all models tested. K = # of model parameters, AICc *w*= AICc weight

Table S6. AICc table for models predicting *Plasmodium elongatum* infection across all age classes.

Models	K	AICc	Δ AICc	AICc *w*
yr+age+wnv+(1|Species)	5	586.2	0.0	0.40
yr+age+wnv+wnv:age+(1|Species)	6	587.0	0.8	0.27
yr+age+(1|Species)	4	587.6	1.4	0.20
mon+yr+age+wnv+(1|Species)	8	590.7	4.5	0.04
mon+yr+age+wnv+wnv:age+(1|Species)	9	591.5	5.3	0.03
yr+wnv+(1|Species)	4	591.9	5.7	0.02
mon+yr+age+(1|Species)	7	592.0	5.8	0.02
age+wnv+(1|Species)	4	594.2	8.0	0.01
age+wnv+wnv:age+(1|Species)	5	594.5	8.3	0.01
yr+(1|Species)	3	595.1	8.9	0.00
mon+yr+wnv+(1|Species)	7	595.5	9.3	0.00
wnv+(1|Species)	3	597.8	11.5	0.00
age+(1|Species)	3	598.1	11.9	0.00
mon+yr+(1|Species)	6	598.9	12.6	0.00
mon+age+wnv+(1|Species)	7	599.9	13.7	0.00
mon+age+wnv+wnv:age+(1|Species)	8	600.2	14.0	0.00
mon+wnv+(1|Species)	6	602.8	16.5	0.00
mon+age+(1|Species)	6	603.7	17.5	0.00
1+(1|Species)	2	603.8	17.6	0.00
mon+(1|Species)	5	609.0	22.8	0.00

Abbreviations: mon= month of capture (4 levels: May/June, July, August, September/October), yr = year of capture (2 levels:2006, 2007), age (2 levels: hatch year juvenile, after hatch-year adult), wnv = WNV serostatus (seropositive, seronegative). Species (13 groups) was a random effect in all models tested. K = # of model parameters, AICc *w*= AICc weight

Table S7. AICc table for models predicting Haemosporida infection across all adult birds.

Models	K	AICc	Δ AICc	AICc *w*
yr+wnv+(1|Species)	4	1138.9	0.0	0.69
mon+yr+wnv+(1|Species)	7	1140.9	2.1	0.24
yr+(1|Species)	3	1144.4	5.5	0.04
mon+yr+(1|Species)	6	1145.5	6.7	0.02
wnv+(1|Species)	3	1157.7	18.9	0.00
mon+wnv+(1|Species)	6	1160.0	21.1	0.00
1+(1|Species)	2	1168.2	29.4	0.00
mon+(1|Species)	5	1169.2	30.4	0.00

Table S8. AICc table for models predicting Haemosporida infection across all juvenile birds.

Models	K	AICc	Δ AICc	AICc *w*
mon+yr+(1|Species)	6	862.7	0.0	0.73
mon+yr+wnv+(1|Species)	7	864.7	2.0	0.27
mon+(1|Species)	5	876.5	13.8	0.00
mon+wnv+(1|Species)	6	878.5	15.8	0.00
yr+(1|Species)	3	892.1	29.4	0.00
yr+wnv+(1|Species)	4	893.9	31.2	0.00
1+(1|Species)	2	904.7	41.9	0.00
wnv+(1|Species)	3	906.4	43.7	0.00

Abbreviations: mon= month of capture (4 levels: May/June, July, August, September/October), yr = year of capture (2 levels:2006, 2007), wnv = WNV serostatus (seropositive, seronegative). Species (13 groups) was a random effect in all models tested. K = # of model parameters, AICc *w*= AICc weight

Table S9. AICc table for models predicting *Plasmodium* infection across all adult (after hatch-year) birds.

Models	K	AICc	Δ AICc	AICc *w*
yr+wnv+(1|Species)	4	871.4	0.0	0.64
mon+yr+wnv+(1|Species)	7	872.6	1.2	0.35
yr+(1|Species)	3	880.6	9.2	0.01
mon+yr+(1|Species)	6	882.4	10.9	0.00
wnv+(1|Species)	3	883.9	12.5	0.00
mon+wnv+(1|Species)	6	886.7	15.3	0.00
1+(1|Species)	2	897.4	26.0	0.00
mon+(1|Species)	5	900.9	29.5	0.00

Table S10. AICc table for models predicting *Plasmodium* infection across all juvenile (hatch-year) birds.

Models	K	AICc	Δ AICc	AICc *w*
mon+yr+(1|Species)	6	785.5	0.0	0.72
mon+yr+wnv+(1|Species)	7	787.4	1.9	0.28
mon+(1|Species)	5	799.9	14.4	0.00
mon+wnv+(1|Species)	6	801.8	16.3	0.00
yr+(1|Species)	3	804.2	18.7	0.00
yr+wnv+(1|Species)	4	806.2	20.7	0.00
1+(1|Species)	2	815.7	30.2	0.00
wnv+(1|Species)	3	817.7	32.2	0.00

Abbreviations: mon= month of capture (4 levels: May/June, July, August, September/October), yr = year of capture (2 levels:2006, 2007), wnv = WNV serostatus (seropositive, seronegative). Species (13 groups) was a random effect in all models tested. K = # of model parameters, AICc *w*= AICc weight

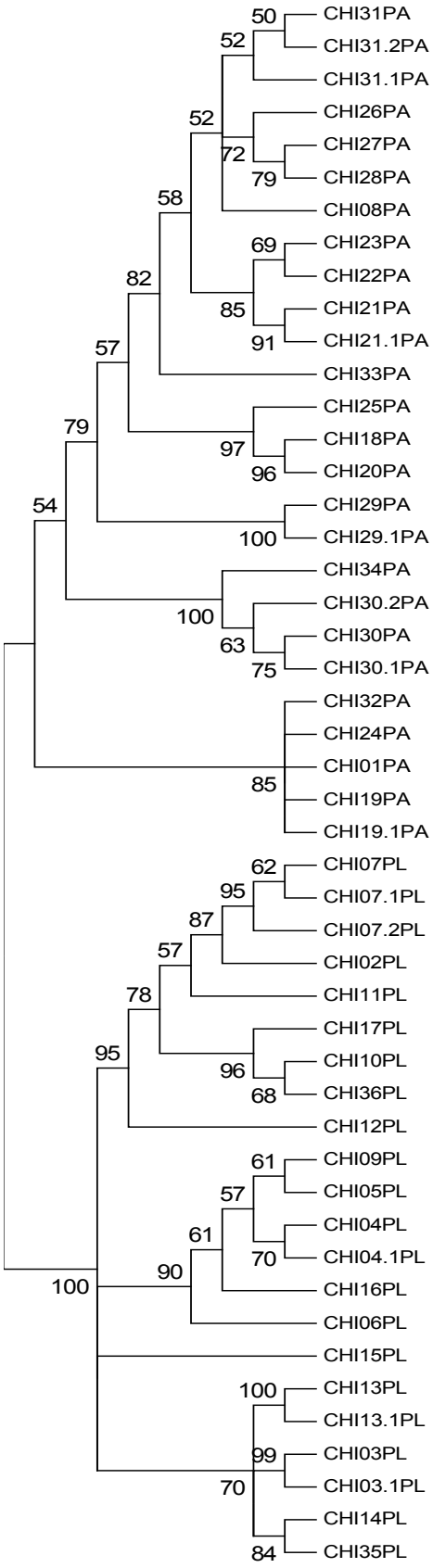

Figure S1. Phylogenetic analysis of all Haemosporida cytochrome *b* lineages by maximum likelihood assuming a GTR+γ substitution model of evolution (γ parameter = 0.3257). Numbers above or below branches indicate bootstrap support (%) estimated from 1,000 resamplings of the sequence data. Branches with ≤ 50% bootstrap support are collapsed. The tree is mid-point rooted. Branch lengths are not drawn to scale. There are 48 unique parasite haplotypes presented here, some of which have been grouped into independent lineages (see Methods for criteria). Lineages denoted by PA are *Haemoproteus,* while those denoted by PL are *Plasmodium*. This tree was primarily used to assign lineages to one of these genera. *Plasmodium cathemerium* and *Plasmodium elongatum* are the lineages CHI03PL and CHI06PL, respectively. Both selection of the substitution model by BIC score and phylogenetic reconstruction were conducted in MEGA5 (Tamura *et al.* 2011).

Section IV. Temporal Analysis of WNV Seroconversion and *Plasmodium* Infection in Avian Hosts

The temporal pattern of WNV and *Plasmodium* transmission in the study site could impact the relationship between WNV serostatus and the probability of a *Plasmodium* infection. For instance, asynchronous transmission dynamics may reduce the overlap of *Plasmodium* positive birds with WNV seropositive birds over a transmission season. This would promote an inverse association between WNV serostatus and *Plasmodium* infection status since one pathogen would be transmitted to hosts before the other. Such a situation may drive an apparent disassociation between WNV and *Plasmodium* without an opportunity for interaction.

We assessed a temporal effect on WNV serostatus and *Plasmodium* infection. Generalized linear mixed models with binomial error distributions and species as a random effect revealed that the probability of having WNV antibodies and *Plasmodium* infection differed across months for adult birds. WNV seroprevalence in adult birds increased across the transmission season (likelihood ratio test of nested models, χ^2=8.6, df=3, p<0.05). WNV seroprevalence was lowest in May-June, moderate in July and August, and highest in September-October. However, the probability of *Plasmodium* infection was consistent across the season (likelihood ratio test of nested models, χ^2=2.6, df=3, p>0.4).

The different patterns of WNV seropositve and *Plasmodium* infected host across the transmission season did not solely drive the negative association between these variables. We repeated the same generalized linear mixed model approach outlined in the main text to a community level dataset that included only adults caught

during the later portion of the transmission season (July through October) when there was more overlap between *Plasmodium*-infected and WNV-exposed individuals. WNV serostatus remained an important negative predictor of *Plasmodium* infection status (Table S11). The estimated WNV serostatus effect (β_{WNV}=-1.72±1.20) indicated that the presence of WNV antibodies reduced the odds of a *Plasmodium* infection by a factor of 5.6 for adult birds.

Table S11. AICc table for models predicting *Plasmodium* infection across adult birds caught during the latter half of the transmission season (July-October).

Models	K	AICc	Δ AICc	AICc *w*
yr+wnv+(1|Species)	4	381.4	0.0	0.52
mon+yr+wnv+(1|Species)	6	381.7	0.3	0.44
wnv+(1|Species)	3	388.5	7.1	0.01
mon+wnv+(1|Species)	5	389.5	8.2	0.01
mon+yr+(1|Species)	5	389.6	8.2	0.01
yr+(1|Species)	3	389.8	8.4	0.01
1+(1|Species)	2	400.3	18.9	0.00
mon+(1|Species)	4	401.3	20.0	0.00

Abbreviations: mon= month of capture (4 levels: May/June, July, August, September/October), yr = year of capture (2 levels:2006, 2007), wnv = WNV serostatus (seropositive, seronegative). Species (13 groups) was a random effect in all models tested. K = # of model parameters, AICc *w*= AICc weight

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Chapter 4

Variation in Prevalence across Hosts Suggest a Trade-off between Generalist and Specialist Avian Haemosporida

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Summary

Parasite specialization evolves despite a reduction in the total number of available hosts. However, specialists are thought to compensate for a reduction in host availability by being more productive on each individual host. Here, we use variation in host breadth among a community of avian Haemosporida to investigate the consequences of generalist and specialist strategies on the distribution of parasite species across hosts. We show that specialist parasites are more common than generalist parasites among shared hosts. Moreover, the density of infections did not vary significantly with host breadth. This suggests that specialists can infect a similar number of hosts as generalists, thus compensating for a reduction in host availability by achieving high prevalence in abundant host species. We also demonstrate that specialist parasites tended to infect older hosts, while infections with generalists were biased toward younger hosts. We suggest that this is associated with differential abilities of generalists and specialists to persist in hosts following infection. Higher prevalence and increased persistence in hosts following infection suggest that specialists are more efficient at utilizing their hosts than generalists, supporting the existence of a trade-off between host breadth and average host-use efficiency among these parasites.

Introduction

Host breadth describes the distribution of a parasite species across host taxa (Poulin 2007). This fundamental trait can vary widely across closely related parasites, from generalists that can infect multiple distantly related host taxa to specialists that are restricted to a single host species (Latta and Ricklefs 2010, Ricklefs *et al.* 2005, Fallon *et al.* 2005, Loiseau *et al.* 2012). The evolution of specialization is challenging because increased specialization results in reduced resource availability (Futuyma and Moreno 1988). For parasites, specialization implies fewer potential host individuals. Explanations for the evolution of specialization generally rely on a tradeoff between the performance of specialists and generalists on their shared hosts, whereby specialists are more adept at utilizing hosts and evading their immune systems (Poulin 1998, Combes 1997). Thus, specialists are predicted to reach greater abundance than generalists on shared hosts, and compensate for reduced host availability by being more productive on each individual host (Poulin 1998, Garamszegi 2006, Straub *et al.* 2011).

Some theoretical and empirical evidence supports a tradeoff between host-use efficiency and host breadth. Theoretical modeling suggests that when a parasite cannot increase its reproductive rate on one host without decreasing it on another, the parasite may evolve a generalist host breadth strategy with intermediate levels of virulence across hosts, or it may specialize on a single host with high virulence. Specialization

is expected more frequently when the cost of host switching is high (Regoes *et al.* 2000). Empirical evidence for relationships between host breadth and host-use efficiency is mixed. Specialized primate malarias (*Plasmodium* sp.) have higher peak parasitemia than generalist malaria (Garamszegi 2006) and specialized aphid parasitoids (subfamily Aphidiinae) and metazoan parasites of fish are more abundant than generalists on their shared hosts (Straub *et al.* 2011, Poulin 1998). However, generalists are more abundant than specialist parasites in some systems. For instance, generalized parasitic trematodes of birds are more prevalent and maintain higher parasitemia than trematodes with narrower host ranges (Poulin and Mouillot 2004). Hellgren *et al.* 2009 used a dataset of European passerines and their *Plasmodium* and *Haemoproteus* parasites to show that generalist parasite taxa can reach higher prevalence within a single host than can specialists. This suggests that some parasites can augment host breadth without forfeiting efficiency in host use, at least among some hosts.

Avian haemosporidian parasites offer a suitable system to explore host breadth strategies. Avian Haemosporida of the genera *Plasmodium* and *Haemoproteus* vary widely with respect to host specialization (Fallon *et al.* 2005, Hellgren *et al.* 2009). Haemosporida have complex life cycles that proceed through an intermediate avian host and a definitive arthropod host. Haemosporida transmission among bird hosts occurs after a dipteran vector ingests infective gametocytes during a blood meal from an infected host. This initiates the sexual phase of the life cycle that culminates in the production of sporozoites that selectively invade the salivary glands of their vector.

Upon a subsequent blood meal, these sporozoites are injected into a host and may result in an infection that leads to the production of gametocytes (Valkiunas 2005). Vectors of Haemosporida are known to have heterogeneous feeding patterns across host species (Malquist *et al.* 2005, Hamer *et al.* 2009, Kilpatrick *et al.* 2006, Santigo-Alacron *et al*. 2012). While this may influence the distribution of parasites by restricting access to certain host taxa (Hellgren *et al.* 2008), analyses of the mosquito vectors of avian *Plasmodium* suggest that vector feeding patterns do not limit parasite distributions across available host species. Instead, host compatibility issues strictly between the parasite and host likely modulate *Plasmodium* host breadth (Gager *et al.* 2008, Medeiros *et al*. 2013), although similar studies are lacking for *Haemoproteus*.

Avian Haemosporida can be pathogenic to their hosts, although health effects can vary across different parasite-host combinations (Palinauskas *et al.* 2011). The course of infection in the avian host varies widely among genera (Valkiunas 2005), however they are generally characterized by an initial acute phase of infection with a peak in parasitemia followed by a chronic phase with relatively low parasitemia (Valkiunas 2005). This chronic phase may last for the duration of the host's life (Atkinson and van Riper 1991, Valkiunas 2005), but longitudinal studies also suggest that individuals can lose infections over time (Latta and Ricklefs 2010, Knowles *et al.* 2011, Wood *et al.* 2013). Infections, even at low chronic levels, have been shown to affect host survival and reproductive success (Lachish *et al.* 2011, Martínez-de la Puente *et al.* 2010, Knowles *et al.* 2010, Merino *et al.* 2000).

The duration of the chronic phase of blood infection may reveal variation in host-use efficiency between generalist and specialist avian Haemosporida. If specialists can more efficiently evade immune defenses, they may persist within hosts longer than generalists. If generalists are less efficient at evading host immune systems, they may be cleared from the host at faster rates than specialists. This could drive a relationship between age and parasite abundance across hosts of specialist and generalist avian Haemosporida. Younger birds have less developed and more naïve immune systems, and presumably would be susceptible to a diversity of parasites, as they have little previous exposure to stimulate memory B-cell formation (Killpack and Karasov 2012, Nemeth and Bowen 2007). Given that exposure to vector-borne disease may occur at young ages (Griffing *et al.* 2007, Hamer *et al.* 2008), young birds likely acquire many infections. If specialists persist in hosts, infections acquired early in life may persist until adulthood and can accumulate with exposure time, driving a positive relationship between prevalence and age. If host immune systems can clear infections of generalist parasites following the acute stage of infection, such parasites would not accumulate with increased exposure time. If transmission is intense for young birds, this may bias generalist infections towards juveniles.

Here, we investigate the consequences of host breadth strategies on the distribution of avian Haemosporida parasites across a community of available host species. We show that within this host community, specialists achieve higher prevalence than generalist haemosporidian parasites on shared avian hosts. Specialists and generalists infect a similar number of host individuals per hectare, suggesting

specialists compensate for the reduction in the total number of available hosts relative to generalists by achieving higher prevalence on abundant hosts. In addition, we show that avian Haemosporida vary in their distribution across host age classes, and that these differences vary with host-breadth strategy. Generalists tended to infect juvenile hosts at higher rates than specialists, while specialists tended to infect adults more than juveniles. We propose that differences in the ability of generalists and specialists to persist in their hosts may drive this effect. Cumulatively, our results support a tradeoff between host-use efficiency and host breadth among a community of haemosporidian parasites.

Methods

Sampling and Parasite Screening

Birds were sampled from 17 locations in suburban Chicago, IL, USA from May-October during 2006-2007. The density of avian host species was estimated through point counts using distance-sampling methods across the same set of sites (see Loss *et al.* 2009 for more information). Birds were captured in mist nets, identified to the species level, and aged by plumage characters where possible. A small blood sample was collected from the jugular vein and preserved in Longmire's lysis buffer for parasite screening. DNA was extracted from this blood sample with a 5M ammonium acetate solution, and purified with an alcohol precipitation. Haemosporidian infections were identified through polymerase chain reaction (PCR)

targeting the 16srRNA of the parasite (Fallon *et al.* 2003). The haemosporidian cytochrome b gene was then amplified from positive samples using a nested PCR protocol (Fecchio *et al.* 2013, Ricklefs *et al.* 2005) and sequenced. While morphospecies are described for avian Haemosporida (Valkiunas 2005), the taxonomy of these parasites is largely unresolved. Some studies suggest that morphospecies may actually be cryptic species clusters (Martinsen *et al.* 2005, 2006). Moreover, some morphospecies may represent distantly related lineages that are difficult to distinguish morphologically (Beadell *et al.* 2006). Presently, evolutionarily lineages of avian Haemosporida are generally identified through unique cytochrome *b* haplotypes (Bensch *et al.* 2000). Here, we recognize haemosporidian taxa as closely related (<1% sequence divergence) monophyletic cytochrome *b* haplotypes recovered from the same set of host species (Ricklefs *et al*. 2005).

Culex mosquitoes, vectors of avian *Plasmodium* lineages (Valkiunas 2005, Kimura *et al*. 2010, Medeiros *et al.* 2013), were sampled among the same sites at the same time birds were being sampled. Mosquitoes were sorted by sex, and females were aggregated into pools of 2-36 individuals, however most pools consisted of 15-25 individuals. DNA was extracted from these pools with Qiagen DNA extraction kits following the manufacturer's protocol. We screened 371 pools of *Culex* mosquitoes for *Plasmodium* parasites following the same protocol as the avian blood samples.

Statistical Analyses

Using the picante package (Kembel *et al.* 2010) in program R, host breadth was estimated as the mean phylogenetic distance between two host individuals drawn at random from the observed distribution of parasites across host species in our sample. Host phylogenetic distances were estimated by the patristic distances of a consensus tree based on 5000 random trees generated from the posterior distribution of a global phylogeny of birds (Jetz *et al.* 2012).

We tested for the relationship between host breadth and prevalence on shared and non-shared host species with a linear mixed model in the lme4 package (Bates *et al.* 2013) in program R. For this analysis, we scored host species as having specialist parasites or not. Hosts of specialized parasites were identified when greater than 90% of infections of a particular parasite lineage were derived from that host species. We fit a model that regressed parasite host breadth, host specialization score, and their interaction on the prevalence of each parasite across each host. Host species and parasite lineage were random effects in the analysis. In addition, we estimated the density of infections (number per unit area) as the sum of the per-host products of parasite prevalence and host density. We then tested for a relationship between infection density and host breadth with linear regression weighted by the square root of the number of infections identified per parasite lineage.

We tested for a relationship between infection status (infected/not infected) and host age class (juvenile or hatch year [HY] bird and adult or after hatch year [AHY] individual) with a generalized linear mixed model assuming a binomial error

distribution for each parasite lineage analyzed here. Host species was a random effect in all models. The model estimates (β_{age}) represent the change in the log-odds of infection for a juvenile host relative to an adult host. Therefore, negative β_{age} estimates indicated that more infections occurred on adult birds, while positive estimates indicated that more infections occurred on hatch-year hosts. We converted the estimates into odds ratios by taking their exponents. We tested for a relationship between these odds ratios and host breadth for each parasite lineage through linear regression weighted by the square root of the number of infections identified per parasite lineage.

Analyses above were limited to parasite lineages in which we had sampled ≥ 20 infections during the study, and host species in which we had sampled at least 10 separate individuals. We set the limit at 20 or more samples for parasite lineages to mitigate problems associated with overfitting age class models which all used at least 2 degrees of freedom. We set the limit at 10 or more sampled individuals per host species because of the general recommendation by Bolker *et al.* (2009) that random effects should have at least 10 individuals per group. Moreover, Jovani and Tella (2006) suggest that prevalence estimates are highly uncertain for samples with fewer than 10 individuals. These criteria were met by 10 parasite lineages and 21 host species.

We investigated avian *Plasmodium* transmission during the study by modeling the infection status of mosquito pools. We used multinomial logistic models in the

nnet package (Venables and Ripley 2002) in program R, and selected among candidate models using AICc in the package AICcmodavg (Mazerolle 2010). The full model included total number of individuals in a pool, month of sampling, year of sampling, and site of sampling as effects. Classes of the dependent variable included infections of 5 *Plasmodium* lineages as well as an uninfected class.

Results

Results of a linear mixed model (Table 1) demonstrate that parasite prevalence increases with parasite host breadth in hosts that do not have specialized parasite lineages. However, the significant negative interaction effect indicated that parasite prevalence decreases with parasite host breadth in host species that have specialized parasite lineages ($p<0.001$, log-likelihood ratio test). These results were driven by the higher prevalence of specialist lineages relative to generalist lineages among shared hosts (Figure 1). This was most evident in the parasite-rich American robin (*Turdus migratorius*; Figure 1a). All four specialized lineages on this host (CHI02PL, CHI04PL, CHI07PL, CHI19PA) had higher prevalence than the four generalist lineages included in the analysis (*Plasmodium cathemerium*, CHI05PL, *Plasmodium elongatum*, CHI18PA). The number of infections per hectare was not significantly related to host breadth ($p = 0.28$), suggesting specialists and generalists infected a similar number of individuals. Indeed, the two best sampled lineages, the generalist *Plasmodium cathemerium* and the robin-specialist CHI07PL, both infected 3.8 hosts per hectare.

Parasites varied in their distribution across age classes. *Plasmodium cathemerium* and *Plasmodium elongatum* were significantly more associated with hatch year birds ($p<0.001$, $p<0.01$ respectively), while CHI02PL, CHI07PL, and CHI08PA were significantly more frequent in adults ($p<0.001$, $p<0.001$, $p<0.05$ respectively; log-likelihood ratio test). The odds ratio of the infection probability in hatch-year hosts relative to adult hosts varied positively with host breadth ($p<0.01$, Figure 2). Thus, generalized parasites were more likely to infect juvenile hosts, while specialists were more likely to infect adult hosts. This effect was present when one considered only the well-sampled American robin, which is typically infected by numerous specialized lineages. Sixteen robin infections represented generalist parasites (CHI05PL, CHI06PL, and CHI03PL). Of these infections, 14 occurred in hatch-year robins. Given that the proportion of hatch-year robins in our sample is 0.55, the probability of 14 or more of 16 coming from hatch-year robins is 0.007.

Variation in the distribution of parasites among hosts of different ages could be driven by annual variation in transmission intensity. To explore this hypothesis, we analyzed *Plasmodium* infections within mosquito vectors. The best fit model explaining infection status in mosquito pools included only month of capture as an effect (AIC *weight*= 0.91), and was differentiated from the next best model which included month and year of capture (ΔAICc= 4.6). Pool size and site of collection were poor predictors of infection status. Overall infection rates were highest in July and August. Different *Plasmodium* taxa peaked at different times. CHI07PL and

Plasmodium cathemerium were generally more common earlier in the season (June-July), while *Plasmodium elongatum,* CHI02PL and CHI04PL were generally more abundant in August (Figure 3). Seasonal heterogeneity raised the possibility that the distribution of *Plasmodium* taxa across age classes could be driven by heterogeneous capture rates of juvenile and adult individuals across months. However, generalized linear mixed models indicated that age remained a significant predictor of *Plasmodium cathermerium*, *Plasmodium elongatum*, CHI07PL, and CHI02PL when both month of capture and host species were included as random effects.

Discussion

Among the Haemosporida parasites included here, specialist parasites are consistently more prevalent than generalists on their shared hosts. In addition, despite the fact that generalists are able to infect more host species, the density of individual infections did not vary significantly with host breadth. Indeed, our sample suggests that specialists and generalists infect similar numbers of individual hosts per unit area. While this is likely associated with high prevalence of specialist among shared hosts, our results also suggest that specialists infect more abundant hosts. For instance, 4 of 6 specialized Haemosporida taxa included here infected American robins, the most abundant native host within the study site (Medeiros *et al.* 2013). Our data suggest that the high prevalence of specialized parasites in an abundant host can compensate for a reduction in the total number or density of hosts available, a fundamental cost of specialization.

Our results contrast with a similar study involving avian Haemosporida among European passerines. Hellgren *et al.* (2009) demonstrated that maximum prevalence of a parasite taxon on a single host species increased with parasite host breadth. The result shows that generalists were able to reach high prevalence on some hosts, even exceeding that of specialists on their host. However, the study did not strictly compare the ability of parasites to utilize shared hosts. This comparison provides a key insight into a trade-off between host utilization efficiency and host breadth among specialists and generalists (Straub *et al.* 2011), since hosts may differ in their overall ability to resist haemosporidian infections. Thus, comparisons of maximum prevalence across different hosts may be confounded.

The prevalence of Haemosporida taxa varied across host age class. Heterogeneous transmission dynamics may affect the distribution of Haemosporida across host individuals (Wood *et al.* 2007), including those of different ages (Waldenstrom *et al.* 2002, Ricklefs *et al.* 2005). For instance, if Haemosporida transmission is epidemic and varies between years, parasite taxa that have high rates of transmission may be expected to occur more evenly among juveniles and adults, while those with waning transmission would occur on adults more than juveniles. However, our data do not suggest that transmission dynamics alone could drive the differences in the distribution of *Plasmodium* taxa across host age class. All *Plasmodium* parasites analyzed here were found among mosquito pools and in hatch-year birds, indicating local transmission. While our survey of *Plasmodium* vectors

extended over only two years, year was a poor predictor of mosquito infection status suggesting that the transmission of *Plasmodium* taxa was similar between 2006 and 2007. Month of capture was an important predictor of *Plasmodium* infection status among mosquito pools, suggesting seasonal heterogeneity in avian malaria transmission. However, including month of capture as a random effect did not change the influence of age class on infection status across the *Plasmodium* taxa analyzed here. Thus, heterogeneity in the sampling effort or catch rate of hatch-year and adult hosts across the season is unlikely to drive the age effects we observed. All together, this suggests that the influence of age class on infection probabilities is driven largely by interactions solely between the host and parasite.

The distribution of parasite taxa among host age class is related to host breadth strategies. The odds ratio of the infection probabilities of juvenile relative to adult hosts varied positively with host breadth. Generalist haemosporidian taxa infected juvenile hosts more often than specialists, while some specialists infected adults more commonly than generalists. Infection dynamics within hosts might underlie this relationship. If haemosporidian parasites chronically infect their host for life, as is widely believed (Atkinson and van Riper 1991, Valkiunas 2005), infections should accumulate with age. However, some studies have demonstrated that infections may disappear from circulation during a host's lifetime, at least for some haemosporidian taxa (Latta and Ricklefs 2010, Knowles *et al.* 2011, Wood *et al.* 2013). In addition, as shown in this study, generalist *Plasmodium* parasites are more prevalent in juvenile hosts. A plausible explanation for the relationship between parasite host breadth and

distribution across host age class may involve differential abilities of specialists and generalists to persist in their host following an acute stage of infection. Specialists should be equally adept at infecting juveniles and adults. Thus, the predominance of specialized infections in adult birds may be associated with the persistence of these infections following initial infection. In contrast, the predominance of generalist lineages in juvenile birds suggests these infections are acquired early but cleared later in life (Ricklefs *et al.* 2005). Alternatively, this distribution across age classes may be the result of differential mortality. Here, infection with specialized Haemosporida may result in higher mortality for younger immunologically naive hosts during an acute infection. These alternative explanations are not mutually exclusive and experimental infections are necessary to further explore these hypotheses.

Increased persistence in a host blood stream would present clear advantages for specialized Haemosporida. Narrow host ranges result in fewer individual hosts to parasitize. The decrease in available hosts may reduce transmission as susceptible hosts become more dilute in the community (Keesing *et al.* 2006). However, the potentially longer duration of blood-borne infection of specialized Haemosporida likely translates into more encounters with vectors over time, and an increase in the number transmission opportunities. This advantage may be key to allowing specialist parasites to infect a similar number of individual hosts compared to generalist parasites within this system, in spite of their narrower host range.

These results support a trade-off between host breadth and average host-use efficiency. Both higher prevalence and increased persistence in hosts suggests that specialized parasites are more efficient than generalists at utilizing shared hosts. Evasion of host immune defenses is central to a tradeoff between host breadth and average host-use efficiency, as immune responses vary across hosts species, and the evolution of evasion mechanisms across multiple host species is assumed to be costly (Combes 1997, Poulin 1998). Increased persistence would suggest that specialized parasites are better able to evade host immune defenses than generalist parasites. Moreover, chronic haemosporidian infections are associated with reduced host survival (Lachish *et al.* 2011, Martínez-de la Puente *et al.* 2010) and thus likely produce disease in their hosts. Increased persistence within the blood stream may indicate specialist Haemosporida are more virulent and exact higher fitness costs on their hosts than generalists (Garamszegi 2006). Ultimately, controlled experimental infections that challenge naïve hosts with specialist and generalist parasites, with close monitoring of the course of these infections, are necessary to fully understand the infection dynamics of generalist and specialist avian Haemosporida. Nonetheless, our study supports a widely argued but infrequently tested constraint on the evolution of host breadth among parasites. Moreover, it highlights the possibility that future study within this system may yield important insights into the evolution of host specialization and its implications for host-parasite interactions.

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Tables and Figures

Table 1. Results of linear mixed model on the relationship between host breadth and prevalence on shared and non-shared host species.

Figure 1. Prevalence of specialized and generalist Haemosporida taxa on hosts with specialized lineages. Specialists, indicated with asterisks, are consistently more prevalent on their hosts than generalist. Lineages ending in "PL" are *Plasmodium*. Lineages ending in "PA" are *Haemoproteus*.

Figure 2. Relationship between host breadth, measured as the mean patristic distance between a pair of randomly drawn host, and the odds ratio of infection of juvenile relative to adult birds. The weighted regression line is solid. Weights are the square root of the number of isolations of the parasite taxa. The font size of the labels is proportional to the weights. The unweighted regression line is dashed.

Figure 3. Distribution of *Plasmodium* taxa in mosquito vectors across months. CHI05PL was isolated only twice and is not included here.

Table 1.

Fixed Effects:	β	Standard Error of β
Intercept	- 0.0055	0.008
Parasite Host Breadth (PHB)	0.0029	0.001
Host Specialization Score (HSS)	0.0505	0.016
PHB*HSS	- 0.0041	0.001
Random Effects:	Variance	Standard Error of Variance
Parasite Lineage	0.00008	0.009
Parasite Species	0.00008	0.009

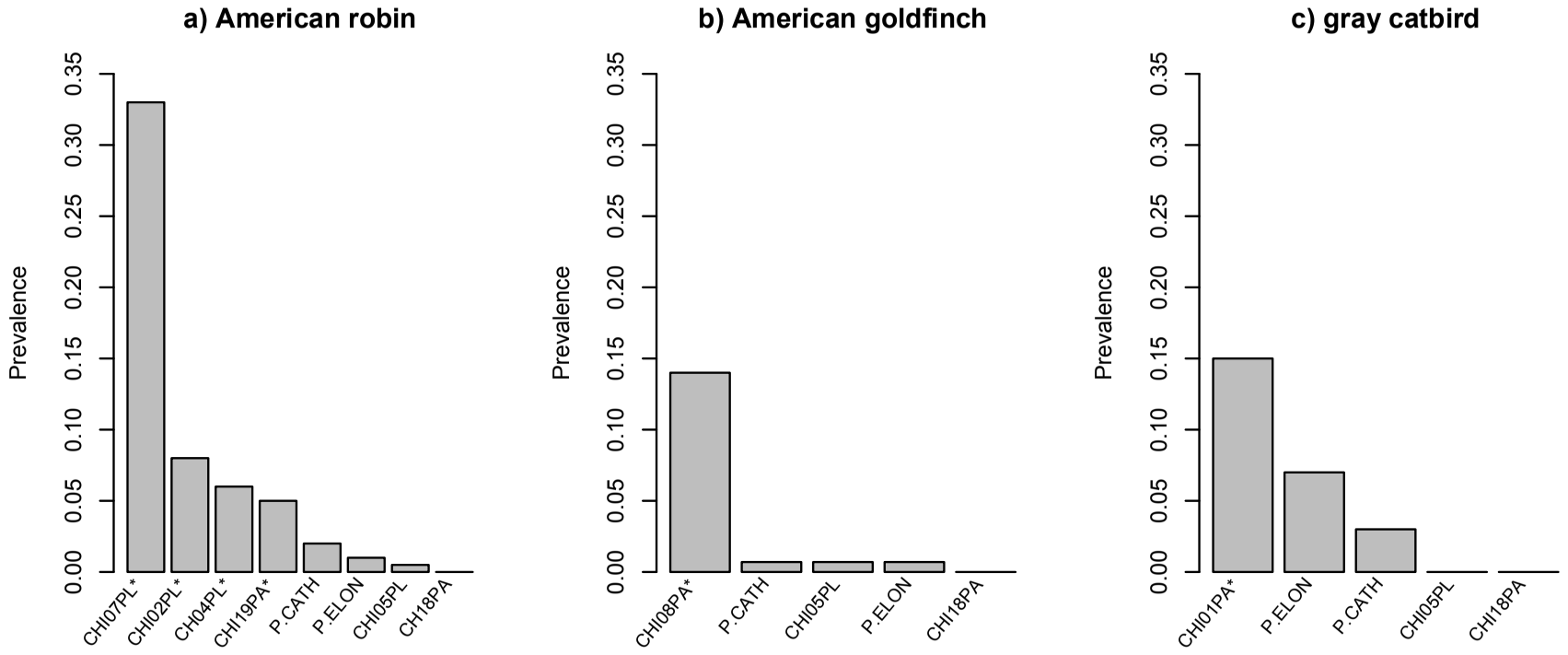

a) American robin
Prevalence
0.00
0.05
0.10
0.15
0.20
0.25
0.30
0.35
CHI07PL*
CHI02PL*
CH04PL*
CHI19PA*
P.CATH
P.ELON
CHI05PL
CH18PA
b) American goldfinch
Prevalence
0.00
0.05
0.10
0.15
0.20
0.25
0.30
0.35
CHI08PA*
P.CATH
CHI05PL
P.ELON
CHI18PA
c) gray catbird
Prevalence
0.00
0.05
0.10
0.15
0.20
0.25
0.30
0.35
CHI01PA*
P.ELON
P.CATH
CHI05PL
CHI18PA

Figure 1.

Figure 2.

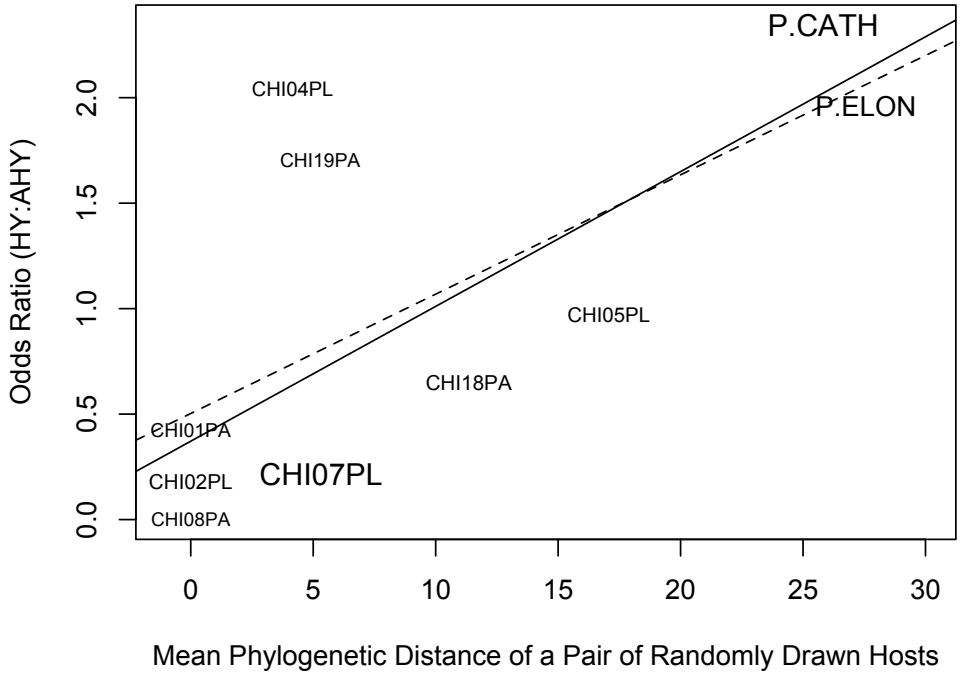
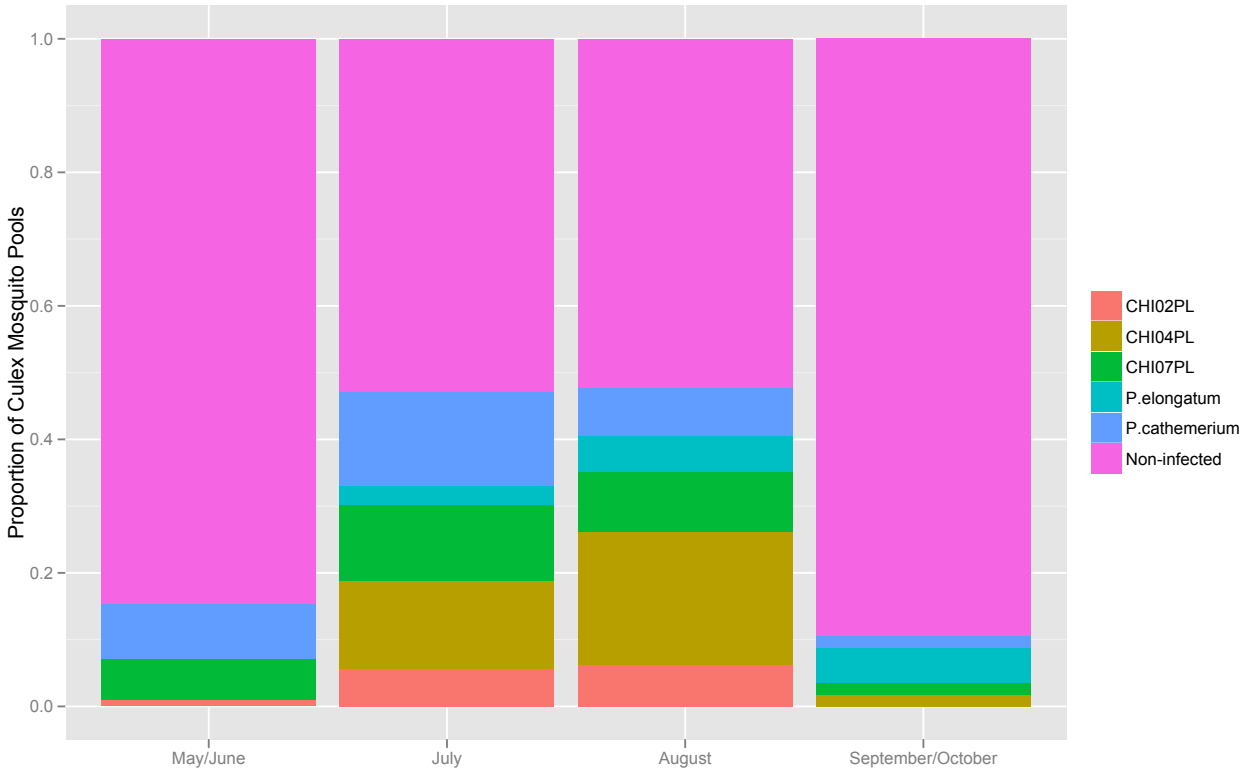

P.CATH
P.ELON
CHI04PL
CHI19PA
CHI05PL
CHI18PA
CHI01PA
CHI02PL
CHI07PL
CHI08PA
Odds Ratio (HY:AHY)
0.0
0.5
1.0
1.5
2.0
0
5
10
15
20
25
30
Mean Phylogenetic Distance of a Pair of Randomly Drawn Hosts

Figure 3.


Proportion of Culex Mosquito Pools
1.0
0.8
0.6
0.4
0.2
0.0
May/June
July
August
September/October
CHI02PL
CHI04PL
CHI07PL
P.elongatum
P.cathemerium
Non-infected