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URBANIZATION AND VECTOR-BORNE DISEASE IN MIGRATORY AND NOMADIC WILDLIFE

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URBANIZATION AND VECTOR-BORNE DISEASE IN MIGRATORY AND NOMADIC WILDLIFE

A DISSERTATION APPROVED FOR THE
SCHOOL OF BIOLOGICAL SCIENCES

BY THE COMMITTEE CONSISTING OF

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ABSTRACT

Vector-borne diseases (VBDs) represented 30% of all emerging infectious diseases at the start of the century (Jones et al. 2008) and pose a devastating global health burden, causing an estimated one million deaths every year. Arthropod vectors and the pathogens they carry are highly sensitive to changes in their environment, and VBD infection dynamics are therefore expected to respond strongly to ongoing anthropogenic impacts on climate and the landscape. Anthropogenic land-use change, such as urbanization, is the most frequently cited influence on the emergence of zoonotic VBDs. Intensifying anthropogenic land-use can increase VBD prevalence by altering the composition of host and vector communities and by exposing hosts to features of urban landscapes that can interact with immunity, such as immunotoxic pollutants and sources of anthropogenic provisioning.

Because infection dynamics of VBDs are intricately linked to vector distributions and environmental conditions, they are uniquely difficult to predict in wildlife taxa that undergo periodic long-distance movements to track seasonal fluctuations in resources. For example, migratory wildlife may be exposed to variable levels of vector activity and urban land-use throughout the distinct breeding, overwintering, and stop-over sites used throughout their annual cycle. In addition, the physiological demands of migration are a source of intrinsic stressors that could interact with extrinsic anthropogenic stressors to shape seasonal infection outcomes. Studying the intersection between animal migration, anthropogenic land-use, and infectious disease is impactful both for the conservation of vulnerable migratory populations and to predict how pathogens might be dispersed to human and wildlife communities across their large geographic range. For example, migratory birds can facilitate the long-distance dispersal of ticks

vectoring Lyme borreliosis, and mediate the spread and enzootic maintenance of West Nile virus after its introduction to the Western Hemisphere.

In this dissertation, I take three distinct but complementary approaches to evaluating how anthropogenic land-use interacts with seasonal wildlife movement to produce vector-borne infection outcomes across ecological and evolutionary scales. Chapter 1 centers on Australian black flying foxes (*Pteropus alecto*), a nomadic nectarivore with winter foraging movements that bring it into increasing contact with anthropogenically modified landscapes and human populations when natural food sources are scarce. Our molecular and phylogenetic work identifies overwintering black flying foxes as hosts of a newly described bacterial VBD closely related to zoonotic *Borrelia* lineages (e.g., the causative agents of Lyme disease in humans), suggesting this species and its ectoparasites warrant further study as potential sources of zoonotic VBDs at the human–wildlife interface in Australia.

In Chapter 2, I focus on temperate migratory songbirds to study how land-use intensity and proximity to spring migration could jointly affect VBDs (haemosporidian parasites and West Nile virus) in New World sparrows wintering in central Oklahoma. I did not find evidence of enzootic West Nile virus activity in overwintering sparrows across 4 sampling years, but both land-use and seasonality predicted birds' infection with haemosporidian parasites. Wintering in urban or suburban habitats overall reduced the probability birds would be infected with any haemosporidian parasite relative to wintering in more natural habitats. I also report evidence that the stationary non-breeding season is a period of active transmission for at least one group of haemosporidian parasites, challenging the paradigm that acute haemosporidian infections do not impact temperate migratory bird populations outside the breeding season.

In Chapter 3, I evaluate the possibility of migration and urban tolerance as host traits that impact patterns of diversification by host-switching in avian haemosporidia by exposing birds to more heterogeneous habitats and their parasite communities over evolutionary time. I found that while urban tolerance did not affect co-evolutionary relationships between hosts and parasites, bird species undergoing long-distance migration had haemosporidian associations that contributed less to global cophylogenetic congruence, suggesting migration drives host-switching events in a common VBD.

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CHAPTER 1

Borrelia lineages adjacent to zoonotic clades in black flying foxes (*Pteropus alecto*), Australia, 2018-2020

INTRODUCTION

Bacteria in the genus *Borrelia* are causative agents of 2 diseases of substantial public health concern: Lyme borreliosis and relapsing fever. Lyme borreliosis is the most frequently reported vector-borne infection in the Northern Hemisphere, and is vectored by ixodid ticks (Khatchikian *et al.*, 2015). By contrast, relapsing fever is globally distributed and vectored predominantly argasid ticks, and thousands of cases of febrile illness in humans are attributed to it annually (Jakab *et al.*, 2022).

Two of the 3 well-recognized monophyletic *Borrelia* clades are the *B. burgdorferi* sensu lato complex that causes Lyme borreliosis and the relapsing fever group that causes relapsing fever; the third clade is a group hosted by reptiles and echidnas (Loh *et al.*, 2017). *Borrelia* from the *B. burgdorferi* s.l. and relapsing fever clades are harbored by a broad range of vertebrate hosts, including birds, reptiles, and mammals. Identifying reservoir hosts and clarifying their role in propagating *Borrelia* are critical for monitoring and mitigating spillover risk. For example, migratory birds can contribute to the long-distance dispersal of *B. burgdorferi* s.l. by transporting millions of ticks within and across continents (Ogden *et al.*, 2008).

Bats might play an underrecognized role in the dispersal and enzootic maintenance of *Borrelia* bacteria. Bats are volant mammals that have been known for more than a century to

harbor borrelial spirochaetes (Nicolle and Comte, 1906), and surveys within the last 5 years indicate bats can host *Borrelia* spp. From the relapsing fever group and from a clade adjacent to *B. burgdorferi* s.l. (Muñoz-Leal *et al.*, 2021; Colunga-Salas *et al.*, 2021). Evidence has shown that bat-associated *Borrelia* infections can be zoonotic because *Borrelia* lineages recovered from bats and bat ticks have been implicated in febrile illness in humans (Qiu *et al.*, 2019). Therefore, the expansion of *Borrelia* research in chiropteran hosts could provide more information about the current and future welfare of both bat and human populations.

Flying foxes (*Pteropus* spp.) represent a group that is highly prominent at the human–wildlife interface in Australia and therefore a target for *Borrelia* bacteria surveillance. The increasing propensity for flying foxes to roost in or near human settlements could increase the overlap of humans and bat ectoparasites and create a potential *Borrelia* bacteria spillover pathway (Eby *et al.*, 2023; Szentiványi *et al.*, 2024). Although black flying foxes (*P. alecto*) have been the subject of extensive research as reservoir hosts of Hendra virus, relatively little study has been devoted to their bacterial communities, including those that could be pathogenic (Szentivanyi *et al.*, 2023). We assessed the presence, diversity, and phylogenetic placement of *Borrelia* spp. Associated with black flying foxes in Australia.

METHODS

As part of ongoing research on the ecology of Hendra virus, we collected blood samples from 840 black flying foxes across 6 sites in southern Queensland and northern New South Wales, Australia, during May 2018–September 2020 (Figure 1.1). We captured bats with mist nets, anesthetized them with isoflurane, and took blood samples preserved on Whatman FTA cards (QIAGEN) until further processing (summarized in Hansen *et al.*, 2022). The Montana State

University Institutional Animal Care and Use Committee (approval no. 201750) and Griffith University Animal Ethics Committee (approval nos. ENV/10/16/AEC and ENV/07/20/AEC) approved the field protocols.

We used QIAamp DNA Investigator Kits (QIAGEN) to extract genomic DNA from blood (four to five 2-mm punches per sample), according to the manufacturer's instructions. To determine *Borrelia* spp. Infection, we used PCR targeting the 16S rRNA gene using primers 1A, 1B, $Bf1_{24}$, and $Br1_{24}$ (Richter *et al.*, 2003) the flagellin (*flaB*) gene using primers FlaLL, FlaRL, 442F, and 442R (Barbour *et al.*, 1996), and the 16S–23S rRNA intergenic spacer (IGS) using primers F, R, Fn, and Rn (Bunikis *et al.*, 2004). Thermal cycler conditions are described in Table 1.1. We purified PCR products within the expected size range for positive results with the Wizard SV Gel and PCR Clean-Up System (Promega) and had the products Sanger sequenced in both directions at Eurofins Genomics. We report prevalence data for IGS, but that gene was not included in phylogenetic analyses because of insufficient reference sequences in GenBank. We manually edited and trimmed the *Borrelia* spp. Sequences from black flying foxes and aligned those sequences with reference sequences in GenBank by using the MUSCLE algorithm in Geneious version 2024.0.5.

We constructed single-marker phylogenies by using both Bayesian and maximum-likelihood methods because too few bat-associated reference sequences of either *flaB* or 16S were available in GenBank to provide an informative concatenated analysis. For Bayesian analyses, we used MrBayes version 3.2 with 10 million Markov chain Monte Carlo generations and a default burn-in of 25%. For maximum-likelihood analyses, we used RaxML version 8.0 using a starting tree obtained by searching for the best-scoring maximum-likelihood tree in a single run and

calculating branch support with 1,000 rapid bootstrap replicates (Stamatakis, 2014). For all trees, we used a general time reversible with a proportion of invariable sites and gamma distribution (GTR+I+G) nucleotide substitution model.

RESULTS

Across our 2-year study period and 6 roosts, 17 (2% [95% CI 1.3%–3.2%]) of 840 black flying foxes tested positive for *Borrelia* spp. By using PCR (Table 1.2). Roost-specific infection prevalence ranged from 0 of 20 samples (no infection found in 3 of the 6 roosts) to 3 (15% [95% CI 5.2%–36.0%]) of 20 samples in Gympie, Queensland, Australia. The roosts with greatest sampling volume were in Toowoomba (402 bats sampled, 3 [0.7%] infected) and Redcliffe (374 bats sampled, 11 [2.9%] infected) in Queensland, Australia (Table 1.3). Most (13/17) infected bats yielded usable sequence data from either 16S rRNA or *flaB* gene targets, which represented 2 haplotypes (p-distances = 8.2% for *flaB* and 1.3% for 16S rRNA). Topologies were similar across tree-building methods but were somewhat discordant between gene targets.

The *flaB* phylogeny grouped black flying fox *Borrelia* spp. With lineages from Macaregua Cave in Colombia in a clade sister to *B. burgdorferi* s.l. (Figure 1.2), but 16S rRNA sequence data are not available from Macaregua Cave as of 2025, and no other sequences from bats in Australia are available. The 16S rRNA phylogeny (Figure 1.3) was also less effective at resolving relationships across the relapsing fever and *B. burgdorferi* s.l. groups supported in previous analyses using multiple markers (Loh *et al.*, 2017). All sequences included here are available at GenBank (accession nos. PQ488732–41 [16S rRNA], PQ492350–60 [*flaB*], and PQ490736–46 [16S–23S IGS]).

CONCLUSIONS

This study corroborated that bats can host *Borrelia* infections evolutionarily distinct from recognized clades. However, the clades are more closely related to *B. burgdorferi* s.l. than the relapsing fever group. We recovered sequences from 11 of 17 infected bats in 2 *Borrelia* spp. Lineages (Table 1.2). Haplotype 1 was represented by a single host that shared a roost in Gympie with a bat infected with haplotype 2, suggesting that variation is unlikely to be structured by geographic isolation. Phylogenetic reconstruction of *flaB* and 16S rRNA suggested *Borrelia* infections from black flying foxes belong to a clade adjacent to existing *B. burgdorferi* s.l. and that relapsing fever groups and are most closely related to *Borrelia* spp. hosted by phyllostomid bats in Colombia (Muñoz-Leal *et al.*, 2021).

Our results are a base for establishing the presence and phylogenetic placement of *Borrelia* infections in flying foxes but underscore research gaps in characterizing their zoonotic potential. Virulence of those lineages in flying foxes is unknown. Although bats are tolerant of multiple viruses, lethal *Borrelia* infections in bats are documented (Irving *et al.*, 2021). The arthropod vector of lineages described in this study also remains unidentified, but host specificity and geographic range of that vector should strongly influence zoonotic risk. For example, certain ixodid bat ticks have generalist feeding habits that could position them as an epidemiologic link between bats, domestic animals, and humans (Evans *et al.*, 2009). Targeted efforts to sample black flying foxes and their ectoparasites across their range are needed to clarify information regarding the zoonotic potential of the novel *Borrelia* lineages described in this study.

TABLES

Table 1.1. PCR primers and reaction conditions used for *Borrelia* sp. PCR testing. All assays used Promega GoTaq Green mastermix (Promega, Madison, WI) and primers at a 0.5 mM concentration. Cycles consisted of initial denaturation at 94°C, followed by the listed number of cycles at 94°C for 30s, listed annealing temperatures for 30s, and extension at 72°C for 30s 16S 1/A/1B primers or 1 minute for 16S $BF1_{24}/BR1_{24}$ and IGS primers.

Target gene	Primer pair	Annealing temperature	Cycles	Amplified fragment length (bp)
16S rRNA	1A/1B	70–61*, 60	10 + 40	≈724
16S rRNA	$BF1_{24}/BR1_{24}$	72–63*, 62	10 + 40	≈1,395
flaB	FlaLL/FlaRL	52	30	≈665
flaB	442f/442r	55	30	≈524
IGS 16S–23S rRNA	F/R	55	30	Variable
IGS 16S–23S rRNA	Fn/Rn	55	30	Variable

*The 16S rRNA screening and extension primers both used a touchdown PCR approach. The annealing temperature was dropped 1°C in each of the first 10 cycles, followed by 40 additional cycles with annealing temperatures as shown in the table.

TABLES

Table 1.2. Metadata on bats PCR positive for *Borrelia* spp. In lineages adjacent to zoonotic clades in black flying foxes (*Pteropus alecto*), Australia, 2018–2020.

Bat ID	Site	Date	Sex	Age	*Borrelia* GenBank accessions
ACGMP001-1	Gympie	2019 Jan	F	Adult	PQ492350,* PQ490736†
ACGMP001-4	Gympie	2019 Jan	F	Adult	PQ492351,* PQ490737†
ACGMP001-5	Gympie	2019 Jan	F	Adult	PQ488732,‡ PQ490738†
ACRED001-15	Redcliffe	2018 May	M	Adult	PQ488733,‡ PQ492352,* PQ490739†
ACRED003-23	Redcliffe	2018 Sep	M	Subadult	PQ488734,‡ PQ492353,* PQ490740†
ACRED004-31	Redcliffe	2018 Dec	F	Adult	PQ488735‡
ACRED006-54	Redcliffe	2019 May	F	Adult	PQ488736,‡ PQ492354,* PQ490741†
ACRED007-51	Redcliffe	2019 Jul	F	Adult	PQ488737,‡ PQ492355,* PQ490742†
ACRED008-54	Redcliffe	2019 Sep	F	Adult	PQ492356,* PQ490743†
ACRED009-72	Redcliffe	2019 Dec	M	Subadult	PQ488738,‡ PQ492357*
ACRED010-32	Redcliffe	2020 Mar	M	Adult	PQ488739,‡ PQ492358*
ACRED010-42	Redcliffe	2020 Mar	F	Adult	PQ488740,‡ PQ490744†
ACRED011-45	Redcliffe	2020 May	F	Adult	PQ492359,* PQ490745†
ACRED011-60	Redcliffe	2020 May	M	Adult	PQ488741,‡ PQ492360,* PQ490746†
ACTOW001-15	Toowoomba	2018 Jun	F	Adult	No sequences
ACTOW002-30	Toowoomba	2018 Jul	F	Subadult	No sequences
ACTOW006-21	Toowoomba	2019 Mar	F	Subadult	No sequences

*Flagellin gene (7lab). †16S–23S rRNA intergenic spacer. ‡16S rRNA.

TABLES

Table 1.3. PCR positivity for Borrelia infections, summarized across *Pteropus alecto* roosts and sampling sessions. Bats are considered infected if testing positive for at least 1 of our 3 markers (i.e., 16S rRNA gene, flaB gene, and 16S–23S ITS rRNA).

Site	Sampling session	Session prevalence	Total no. sampled	Site prevalence
Gympie	31 January 2019	0.20	20	0.20 (4/20)
Hervey Bay	15 July 2018	0	6	0
Hervey Bay	28 July 2020	0	30	
Maclean	9 July 2018	0	1	0
Mt. Ommaney	17 January 2019	0	7	0
Redcliffe	25 May 2018	0.04	24	0.03 (10/375)
Redcliffe	27 July 2018	0	10	
Redcliffe	14 September 2018	0	19	
Redcliffe	14 December 2018	0.05	21	
Redcliffe	8 March 2019	0	59	
Redcliffe	28 May 2019	0.03	30	
Redcliffe	9 July 2019	0.03	32	
Redcliffe	10 September 2019	0.03	30	
Redcliffe	3 December 2019	0.03	32	
Redcliffe	3 March 2020	0.07	30	
Redcliffe	11 May 2020	0.07	30	

Table 1.3 (*continued*).

Site	Sampling session	Session prevalence	Total no. sampled	Site prevalence
Redcliffe	7 July 2020	0	30	
Redcliffe	7 September 2020	0	28	
Toowoomba	3 June 2018	0.05	21	0.007 (3/402)
Toowoomba	21 July 2018	0.04	26	
Toowoomba	8 September 2018	0	21	
Toowoomba	8 December 2018	0	22	
Toowoomba	11 January 2019	0	9	
Toowoomba	15 March 2019	0.02	58	
Toowoomba	14 May 2019	0	30	
Toowoomba	2 July 2019	0	29	
Toowoomba	23 July 2019	0	6	
Toowoomba	3 September 2019	0	30	
Toowoomba	10 December 2019	0	30	
Toowoomba	10 March 2020	0	29	
Toowoomba	4 May 2020	0	30	
Toowoomba	14 July 2020	0	30	
Toowoomba	1 September 2020	0	30	

FIGURES

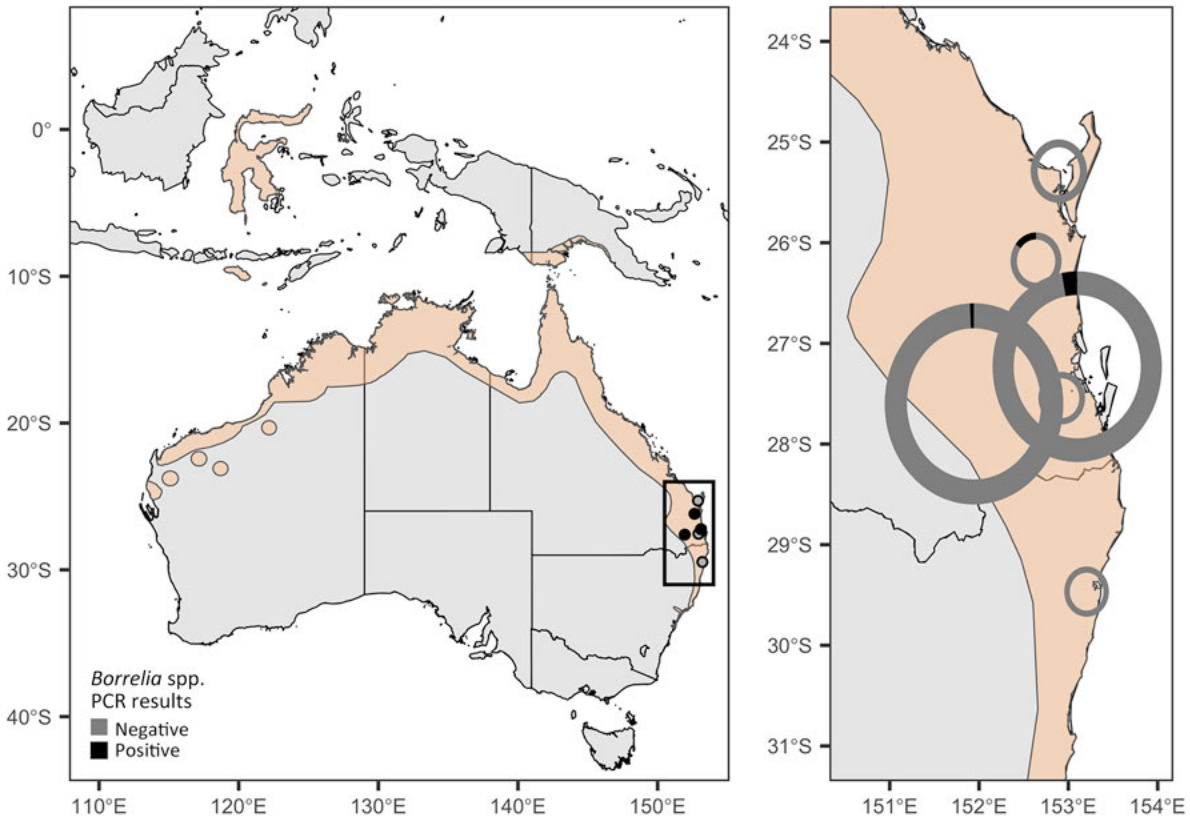


Figure 1.1. Sampling sites in eastern Australia (Queensland and New South Wales) of *Borrelia* lineages adjacent to zoonotic clades in black flying foxes (*Pteropus alecto*), Australia, 2018–2020. *P. alecto* geographic distribution (beige shading) is shown as defined by the International Union for Conservation of Nature. Circles are colored by the presence or absence of *Borrelia* spp. infections detected in blood samples from bats at each site. Black box at left indicates area enlarged at right, showing donut plots with the fraction of PCR-positive samples in black; circles are scaled by sample size.

FIGURES

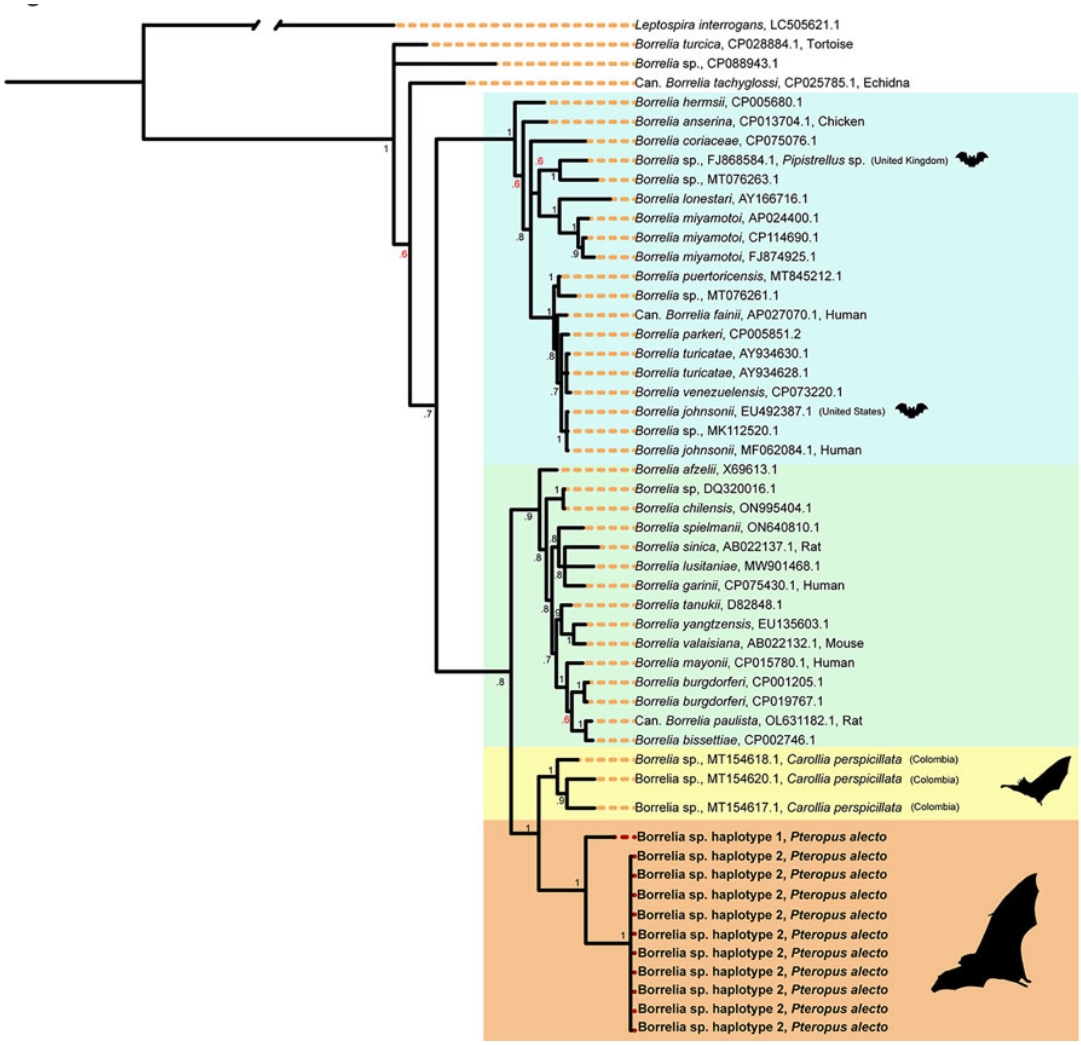


Figure 1.2. Bayesian phylogenetic tree of *flaB* gene, constructed with a GTR+I+G substitution model and 10 million MCMC generations. Colors represent major *Borrelia* groups and clades of interest: blue, relapsing fever group; green, *Borrelia burgdorferi* sensu lato complex; yellow, *Borrelia* spp. from Macaregua Cave, Colombia; light orange, new *Borrelia* haplotypes from black flying foxes in Australia. Red node text represents posterior probabilities <0.70. Host associations are noted when GenBank lineages were isolated from vertebrates, barring experimental infections. Lineages associated with bats or bat ticks are marked with a bat graphic (sourced from Noun Project).

FIGURES

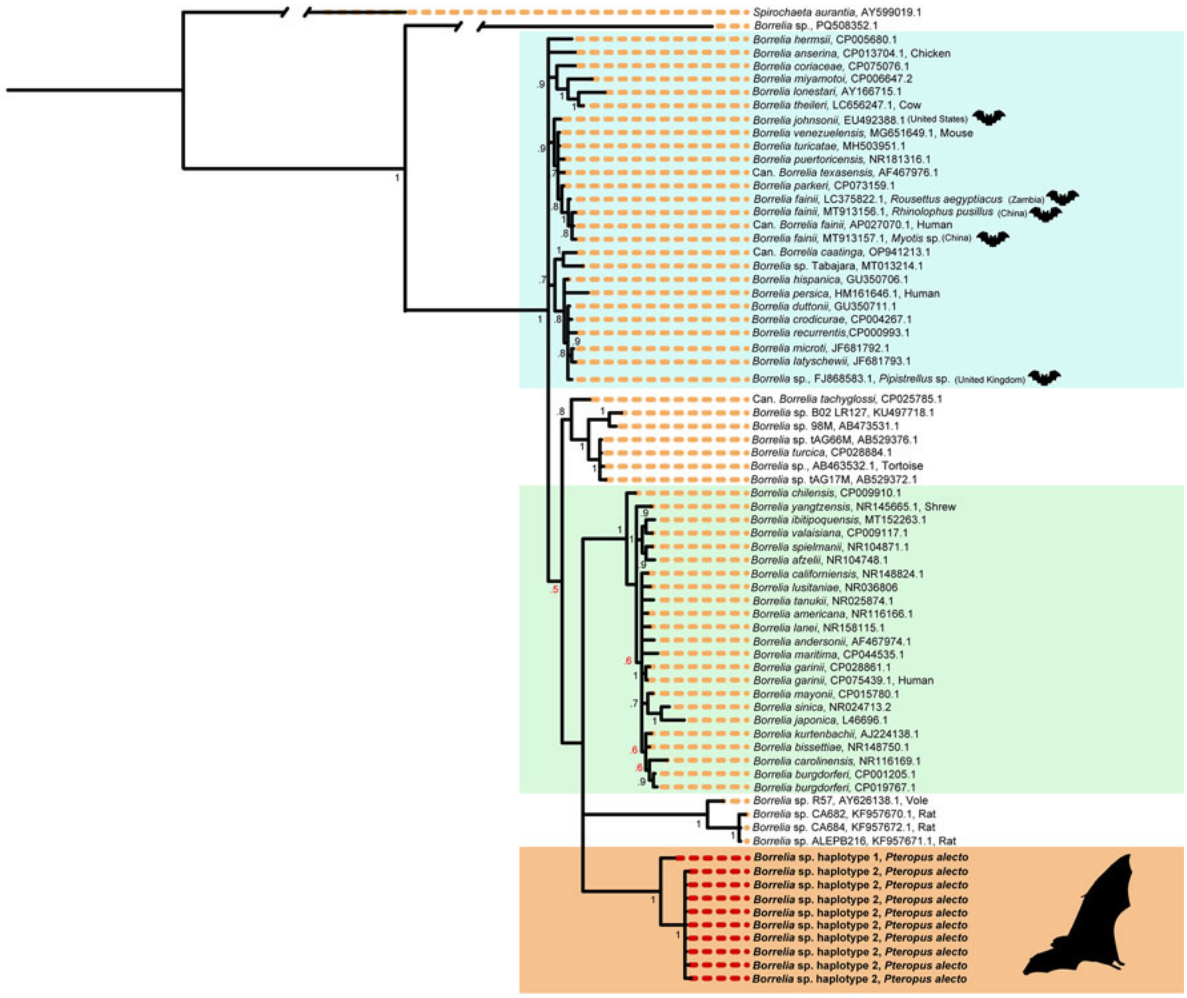


Figure 1.3. Bayesian phylogenetic tree of 16S rRNA gene, constructed with a GTR+I+G substitution model and 10 million MCMC generations. Colors represent major *Borrelia* groups and clades of interest: blue, relapsing fever group; green, *Borrelia burgdorferi* sensu lato complex; yellow, *Borrelia* spp. from Macaregua Cave, Colombia; light orange, new *Borrelia* haplotypes from black flying foxes in Australia. Red node text represents posterior probabilities <0.70. Host associations are noted when GenBank lineages were isolated from vertebrates, barring experimental infections. Lineages associated with bats or bat ticks are marked with a bat graphic (sourced from Noun Project).

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CHAPTER 2

Anthropogenic land-use during the overwintering season alters vector-borne infection dynamics in a temperate songbird community

INTRODUCTION

The loss and degradation of natural landscapes wrought by anthropogenic change is threatening biodiversity at a global scale, with future urban expansion confronting 34% of threatened terrestrial vertebrates with local extinction (Li *et al.*, 2022). Anthropogenic threats like urbanization are especially detrimental to migratory wildlife, which have lives organized into annual cycles with large geographic ranges, dynamic habitat requirements, and overlapping threats (Hardesty-Moore *et al.*, 2018; Cooke *et al.*, 2024). Animal migration is critical to ecosystem function, as migrants may seasonally influence trophic structures across multiple communities and aid in the long-distance dispersal of seeds and symbionts (Bauer and Hoye, 2014). However, migratory populations are in relatively steep decline because their habitat requirements can span vast distances and geographic borders (Wilcove and Wikelski, 2008). Birds represent the best-studied migratory taxa in urban ecology and have significantly shaped our understanding of anthropogenically mediated population declines (Marzluff, 2017). Migratory bird species experiencing the most dramatic losses are often ecological specialists excluded from urbanized habitats, but even generalist "urban-tolerant" species that are seemingly better-equipped to cope with anthropogenic change by using modified habitats for one or more stages of their annual cycle are not shielded from population decline (Petrenko *et al.*, 2024). Traits conferring urban tolerance may not have a protective effect on avian biodiversity, because

migratory birds using urbanized habitats are exposed to a host of anthropogenic threats including building collisions, reduced food quantity or quality, and infectious disease (Bradley and Altizer, 2007; Cooke *et al.*, 2024).

Multiple aspects of urban environments can have profound consequences for health and pathogen transmission in wild birds. Wildlife communities in cities can contain lower species richness and favor dense populations of synanthropic species (Evans *et al.*, 2011; Aronson *et al.*, 2014), hampering the potential role of diversity in reducing disease risk by distributing infection opportunities across a heterogeneous pool of competent and non-competent hosts (i.e., the dilution effect). The altered community compositions in urban landscapes can therefore accelerate disease outbreaks if thriving species are also highly competent hosts (Kilpatrick *et al.*, 2006). In addition, resources in urban landscapes are frequently more clumped and predictable across space and time than resources in natural landscapes, increasing contact rates within and between species. The epidemic spread of *Mycoplasma gallisepticum*, a bacterial pathogen causing conjunctivitis in House Finches across North America, has been linked to the aggregation of overwintering flocks at bird feeders (Altizer *et al.*, 2004; Adelman *et al.*, 2015). Bird feeders may have similarly enabled the emergence of *Trichomonas gallinae*, a protozoan parasite causing devastating outbreaks of trichomonosis in Greenfinch and Chaffinch populations in Europe, by encouraging contact between the columbid hosts it co-evolved with and other granivorous species (Lawson *et al.*, 2011, 2018). In addition to altering host contact patterns, using urbanized habitats can affect within-host processes that determine susceptibility to infection (Minias, 2023). Common urban pollutants such as heavy metals, noise, and artificial nighttime light can suppress immune function in wild birds (Dominoni *et al.*, 2016; Chatelain *et al.*, 2016; Brumm *et al.*, 2021). Bird feeders and other sources of anthropogenic provisioning

common across urban landscapes can also have divergent, context-dependent effects on immunity and infection outcomes (Becker and Hall, 2014). Abundant urban food sources may improve body condition and enable birds to better invest in immune defense; however, if urban birds are exploiting poor-quality and nutrient-deficient anthropogenic resources, immunity can instead be impaired (Cornet *et al.*, 2014; Murray *et al.*, 2018; Strandin *et al.*, 2018).

Despite these complexities of contact-transmitted infections such as conjunctivitis and trichomonosis, predicting the effect of urbanization on pathogens transmitted by arthropod vectors can be even more challenging. Vector community composition is a strong predictor of infection dynamics (Ferraguti *et al.*, 2018; Guo *et al.*, 2019), and increased host aggregation does not necessarily increase vector exposure (Janousek *et al.*, 2014). Vector-borne transmission can only occur in environments that support both suitable hosts and arthropod vectors, the latter of which are highly sensitive to thermal and microhabitat differences in urban landscapes (LaDeau *et al.*, 2015). For example, urban habitats can be inhospitable to arthropod vectors with specialized habitat requirements, disrupting the life cycle of vector-borne pathogens and decreasing infection prevalence (Werner and Nunn, 2020). Other vector species might benefit from modified landscape features such as urban heat island effects, which can increase vector performance (i.e., development, biting rate) and alter seasonal activity windows (Townroe and Callaghan, 2014; Mordecai *et al.*, 2019). Like urban bird communities, urban vector assemblages also tend to be less diverse and to be dominated by a core of urban-tolerant species that can vector pathogens with high public health burdens (Perrin *et al.*, 2022; Skinner *et al.*, 2023; Bauer *et al.*, 2024).

In addition to changes in vector communities and thereby exposure, aforementioned changes in host immunity from urbanization could especially shape vector-borne infection

outcomes. Immunotoxic trace metals in urban environments can increase the prevalence of vector-borne haemosporidian parasites in wild songbirds (Bichet *et al.*, 2013; Werner *et al.*, 2026). Artificial nighttime light is another immunosuppressive and ubiquitous urban pollutant that can be linked directly to the within-host dynamics of vector-borne disease risk (Kernbach *et al.*, 2019; Becker *et al.*, 2020). The exposure of wild birds to artificial nighttime light can not only increase susceptibility to infection and lengthen the period a bird is infectious to naive vectors (Kernbach *et al.*, 2018, 2019), but also cause the reactivation of chronic infections and in tandem with normal seasonal processes, such as migratory relapse. Migratory relapse refers to relapse events caused by dampened immunity as migrants trade-off relatively non-essential functions like immune defense to prepare for and undergo long-distance movement (Gylfe *et al.*, 2000; Owen and Moore, 2008; Tobolka *et al.*, 2024). A study on captive Dark-eyed Juncos (*Junco hyemalis*) found that migratory individuals experienced more intense spring and fall relapse of chronic haemosporidian infections when exposed to artificial nighttime light during these periods (Becker *et al.*, 2020). Such work suggests an interaction between urban and seasonal stressors in shaping vector-borne infection outcomes in migratory bird populations, representing a new challenge for mitigating anthropogenic threats to migratory species: understanding how pathogens differentially impact migratory bird populations overlapping with urbanized habitats will require studying these threats in the broader context of their annual cycle.

We here test the hypothesis that migratory birds are disproportionately vulnerable to new and relapsing vector-borne infections when they use urbanized habitats during sensitive parts of their annual cycle (i.e., periods marked by suppressed immunity during energetically costly life-history events such migration and/or increased exposure to arthropod vectors). We focus on the

non-breeding (i.e., overwintering) season, a portion of the annual cycle that receives relatively little attention both in general research of migration ecology (Marra *et al.*, 2015) and in the context of vector-borne disease in temperate systems, as arthropod vectors are assumed to be dormant (Dunn *et al.*, 2013). We posit that overwintering could be a surprisingly important epidemiological window for several reasons. First, the non-breeding season is the period when migratory birds have the least specialized habitat requirements and are most likely to inhabit urbanized landscapes (Callaghan *et al.*, 2021). Second, birds that contract new or relapsing vector-borne infections prior to fall migration during the breeding season are likely to meet an epidemiological dead-end when vectors are absent at the wintering ground, whereas contracting infections at the non-breeding grounds, or experiencing relapse of chronic infection, just prior to spring migration would position hosts for onward transmission to an emerging vector community during stopover or upon arrival at the breeding grounds. Finally, the assumption that arthropod vectors are dormant during the overwintering period is weakened closer to spring migration. This is particularly applicable to vector taxa known to emerge in early spring in temperate regions, such as biting midges (Valkiunas, 2004). However, the phenological advancement of vectors that emerge in late spring and summer months is also a mounting concern in an increasingly warming world (Morin and Comrie, 2013; Hall *et al.*, 2016).

We test this hypothesis using two vector-borne disease systems in wild birds: avian haemosporidia and West Nile virus (WNV). Avian haemosporidia are globally distributed and hyperdiverse protozoa widely used as a model system for studying eco-evolutionary relationships between hosts, vectors, and pathogens (Dunn and Outlaw, 2019). Haemosporidian infections span three genera with ecologically and phenologically distinct groups of arthropod vectors. The genus *Plasmodium* is vectored by culicid mosquitoes, *Haemoproteus* is vectored by

ceratopogonid biting midges and occasionally hippoboscid flies, and *Leucocytozoon* is vectored by black flies (Valkiunas, 2004). Haemosporidians also have well-defined latency–reactivation dynamics, wherein infection intensity in host peripheral blood can fluctuate over time (Beaudoin *et al.*, 1971; Pigeault *et al.*, 2023). The acute phase of infection is marked by high intensity (e.g., increased numbers of circulating gametocytes), followed by a chronic, low-intensity phase that can persist in birds for months or years (Valkiunas, 2004). Chronic haemosporidian infections can re-enter acute phases in response to intrinsic and extrinsic stressors that weaken host immunity (Beaudoin *et al.*, 1971; Becker *et al.*, 2020). The impacts of haemosporidian infections on their avian hosts are diverse, ranging from sublethal negative impacts on individual fitness (Grabow *et al.*, 2025) to aggressive mortality with population- and species-level consequences (Woodworth *et al.*, 2005; Dadam *et al.*, 2019).

In contrast, WNV is exclusively vectored by mosquitoes and has a single, brief infectious period in avian hosts (1–7 days; (Komar *et al.*, 2003). WNV is a widespread zoonotic flavivirus responsible for over a thousand annual cases of neuroinvasive disease in the United States (McDonald *et al.*, 2021). WNV risk tracks both measures of urbanization, which can provide favorable conditions for its vectors, and the distribution of migratory bird hosts. Some passerines are highly competent reservoirs of WNV, facilitating enzootic transmission cycles that cemented the virus's permanent establishment in the Western hemisphere (Kilpatrick, 2011; Tolsá *et al.*, 2018). Overwintering is the least-understood part of the WNV transmission cycle, but chronic WNV infections in overwintering migratory birds followed by spring relapse to infect emerging vectors may be a mechanism by which WNV persists across years (Reisen *et al.*, 2006b). Overwinter WNV seroconversion in a resident bird species has been documented at least once in the Great Plains region (Shelite *et al.*, 2008). Low levels of year-round transmission can also

occur in regions where dominant WNV vectors do not enter a fixed diapause for harsh winters and might emerge on warm days irrespective of season (Tesh *et al.*, 2004), and winter temperatures can accordingly be important predictors of interannual variability in human WNV cases (Wimberly *et al.*, 2014).

We examined associations between haemosporidian and WNV infection and urbanization in an overwintering bird community in central Oklahoma. We targeted migratory sparrows (Aves: Passerellidae), a group well-represented by habitat generalist species found across a gradient of urbanization in the Great Plains region. We repeatedly sampled passerellid assemblages using urban, rural, and suburban habitats from mid-winter (January) until spring migration to their breeding grounds (May). This sampling scheme allowed us to test the hypothesis that vector-borne infection outcomes (i.e., haemosporidian positivity and intensity, WNV positivity and seropositivity) increase with urbanization of overwintering habitat and with proximity to spring migration.

METHODS

Study area

We captured overwintering wild sparrows across six sites in central Oklahoma between 2021 and 2025. We initiated annual sampling seasons in January and stopped in early May, following the relatively complete spring migration of focal species from Oklahoma. We sampled at two site replicates for each of three land-use categories (urban, suburban, or rural), which were quantitatively differentiated using the global human modification index (GHM; Kennedy *et al.*, 2020). The GHM aggregates the distribution of 13 anthropogenic stressors across the terrestrial biosphere (including human population density, proportion of built-up and agricultural areas, and artificial nighttime light levels) and weighs the relative intensity of each stressor to produce a

metric between 0 (pristine habitat) and 1 (wholly human–modified landscape). We extracted GHM indices from each sampling site within the estimated 30-hectare (0.3 km^2) estimated wintering home range of Dark-eyed Juncos as our most representative target species (Gottfried and Franks, 1975); Figure 2.1). Our urban site replicates were two urban greenspaces in Oklahoma City, Oklahoma: Martin Park Nature Center (MPNC) and Will Rogers Gardens (WRP; Figure 2.1). Both urban sites contained permanently or intermittently maintained bird feeders at the time of sampling, and are each bordered by a major highway and large residential areas. Suburban site replicates in Norman, Oklahoma were Ruby Grant Park (RGP), a manicured walking trail with pockets of suitable sparrow habitat surrounded by neighborhoods, and a brushy area bordering Oliver's Woods (OW), which is adjacent to the University of Oklahoma campus and residential areas. Rural site replicates were within Lexington Wildlife Management Area (LEX) and Kessler Atmospheric and Ecological Field Station (KAEFS), public or university-managed land both south of Norman in sparsely populated areas. The closest pair of site replicates was 12 km apart, and no individuals were resighted or captured at multiple sites.

Sample collection

We rotated sampling sites according to current sample size and habitat category to ensure habitat categories were as evenly represented across winter and spring seasons as possible. During each sampling session, we captured birds in 38 mm mist nets (Avinet) and temporarily held birds in individual paper bags prior to processing. Sampling sessions spanned about 5 hours after sunrise, during which mist nets were checked every 15 minutes. For each bird, we applied unique aluminum bands for individual identification (U.S. Fish & Wildlife Service) and recorded standard morphometric measurements (unflattened wing chord, tail length, and "fat score", or the visible deposition of subcutaneous fat). Where possible, we also aged birds by molt and sexed by

plumage according to Pyle (1999). We collected no more than 150 µL blood from each bird (constituting <1% of body mass) by lancing the brachial vein with a sterile 27G needle and collecting blood with heparinized capillary tubes. We immediately prepared thin blood smears for hematology and partitioned the remaining blood into 700 µL DNA/RNA Shield (Zymo) for downstream molecular analyses as well as used centrifugation on remaining sample to separate plasma for WNV antibody detection. Blood in DNA/RNA Shield and plasma were stored at -80°C at the University of Oklahoma (OU). All birds were processed within two hours of capture then released at the field site. Sampling sessions strongly prioritized migratory passerellids, but we opportunistically sampled other passerines where possible. Bird capture and sample collection were approved by the Institutional Animal Care and Use Committee of OU (R21-026, 2022-0199), the Oklahoma Department of Wildlife Conservation (10567389), and a Federal Bird Banding Permit (24394).

Molecular detection of haemosporidians

We extracted genomic DNA from blood preserved in DNA/RNA Shield using the *Quick*-DNA Miniprep Plus kit (Zymo), following the manufacturer's instructions. We used PCR to amplify a 479 bp fragment of the cytochrome *b* gene, the most widely applied region for detecting and describing the genetic diversity of haemosporidians (Bensch et al. 2009). We tested each sample with the three primer pairs described in Drovetski et al. 2014, which combine a single forward primer (UNIVF) with one of three reverse primers (UNIVR1, UNIVR2, or UNIVR3) to target haplotype diversity spanning the genera *Plasmodium*, *Haemoproteus*, and *Leucocytozoon*. Reactions consisted of 8.5 µL nuclease-free water, 2 µL each of forward and reverse primer diluted to 10 uM, 2 µL DNA template, and 12.5 µL GoTaq Green Mastermix (Promega). Thermal cycler settings are outlined in Table S1. Each set of reactions included a positive control

(haemosporidian infection confirmed via microscopy and/or previous PCR) and negative control (nuclease-free water). We tested all negative samples twice to limit false negatives.

Sanger sequencing was conducted at North Carolina State University Genomics. We aligned forward and reverse sequences and cleaned sequence data by eye in Geneious Prime 2024.0.05. We assigned recovered haplotypes to known haemosporidian lineages by BLASTing sequences to the MalAvi database (accessed 2/25/2025).

Microscopic detection of haemosporidians

We air-dried and stained blood smears with Wright–Giemsa (Quick III Set, Astral Diagnostics Inc.). To quantify haemosporidian infection intensity, we counted the number of haemosporidians present in approximately 40,000 erythrocytes. Specifically, we identified a "standard" field of view (FoV) containing a uniform monolayer of erythryocytes, determined this standard FoV should contain approximately 400 erythryocytes, then counted gametocytes in 100 FoVs visually consistent with the standard (Dadam *et al.*, 2019). We identified active infections to genus based on gametocyte morphology (Valkiunas, 2004). The same observer (TBV) screened all blood smears.

Molecular detection of WNV

Total RNA was extracted from blood preserved in DNA/RNA Shield using the *Quick*-RNA Viral kit (Zymo), following the manufacturer's instructions. WNV RNA was detected via qRT-PCR with 5ul input RNA using the EXPRESS One-Step qRT-PCR kit and WNV-specific primers and probes that amplify the envelope coding region as previously described (Gallichotte *et al.*, 2024; Table S2.1). Positive and negative extraction controls and RNA standards were included.

WNV serology

Plasma samples were heat inactivated at 56°C for 30 minutes. A standard plaque reduction neutralization test (PRNT) was performed as previously described (Gallichotte *et al.*, 2024). Briefly, plasma was mixed with approximately 40 WNV plaque forming units and incubated at 37°C for 1 hour. Samples were added to confluent Vero cells, incubated for an additional hour, then overlaid with a semi-solid media and incubated for 3 days. Cells were fixed and stained with ethanol, and plaques counted manually. Samples with greater than 50% neutralization at a final plasma dilution of 1:20 were considered seropositive.

Comparison of WNV molecular detection

To determine the most sensitive detection method of WNV RNA in bird blood, we compared traditional end-point PCR targeting the NS5 coding region or sfRNA (5' UTR), and a real-time qRT-PCR targeting the envelope coding region (described above; Table S1), using previously published RNA from viremic experimentally WNV-infected American robins (Gallichotte *et al.*, 2024). qRT-PCR was performed as described above. For end-point PCR, cDNA was generated using ProtoScript II following manufacturer instructions with 7µl RNA in a 20ul reaction volume. PCR was performed with GoTaq Green, with 5µl input RNA (same as qRT-PCR), in 25ul reaction volume. Thermocycling conditions were an initial denature at 94°C for 30 seconds, then an additional 30 sec denature at 94°C, anneal for 30 sec at 54°C for the sfRNA primers and 52°C for NS5 primers, and 30 sec extension at 68°C for 35 cycles total, followed by a 5 minute final extension at 68°C. PCR products were run on a 5% agarose gel and DNA visualized using SYBR-Safe gel stain. Across three bird samples, a larger range of viremia was detected using qRT-PCR, followed by NS5. Primers targeting sfRNA only detected products at peak viremia.

Statistical analyses

We conducted all statistical analyses in R 4.5.2. To test if the effect of land-use intensity (rural, suburban, or urban) on vector-borne infections were mediated by season (ordinal sampling date), we used generalized linear mixed-effect models (GLMMs) as implemented in the *lme4* and *glmmTMB* packages. We considered four haemosporidian infection outcomes as response variables: overall infection positivity (presence of any infection identified using PCR; binomial response), active infection positivity (presence of transmissible infections containing at least one gametocyte in the blood smear; binomial response), infection intensity (number of infected erythrocytes per 100 FoV, i.e. per 40,000 erythrocytes; zero-inflated, beta-distributed response), and within-host infection richness (number of unique haemosporidian haplotypes recovered from each individual; truncated Poisson response). The latter two sets of analyses were restricted to only PCR-positive birds. All models included habitat and season as well as random effects for host species and for site nested within year. We also included host body condition as a fixed effect in all models by regressing mass by wing chord and calculating residuals separately by host species (mass~wing chord + wing chord|species). We used maximum likelihood (ML) and Akaike information criterion (AIC) to compare base models with additive effects against analogous models with a three-way interaction between habitat, season and migration timing or only an interaction between habitat and season (Burnham and Anderson, 2002). We considered models within two ΔAIC to be competitive and refit the most parsimonious model using restricted ML (REML) for statistical inference. We assessed fit using both marginal and conditional R^2 (R^2_m and R^2_c; Nakagawa *et al.*, 2017). We used *emmeans* to derive marginal means and odds ratios (ORs) and adjusted *p*-values for pairwise contrasts for the inflated false discovery rate. Where possible, each analysis was also stratified by haemosporidian genus, given the three

genera have distinct arthropod vectors and transmission windows that could moderate the effects of land-use and season on infection outcomes.

Because of their larger sample size, positivity models additionally included a categorical fixed effect for spring migration timing to account for phenological differences, which could shift the timing of migratory relapse and influence overlap with arthropod vectors. We used eBird weekly bar charts (Sullivan *et al.*, 2009) during the study period (2021–2025) to distinguish between host species that had mostly completed spring migration from Oklahoma by April (early migrants) and species that did not migrate from Oklahoma until May (late migrants; Figure S2.1). The early migrant group comprised Dark-eyed Juncos, Fox Sparrows (*Passerella iliaca*), and Song Sparrows (*Melospiza melodia*), whereas the late migrant group comprised Harris's Sparrows (*Zonotrichia querula*), White-throated Sparrows (*Zonotrichia albicollis*), White-crowned Sparrows (*Zonotrichia leucophrys*), and Lincoln's Sparrows (*Melospiza lincolnii*). We then used ML and AIC to compare models with all additive effects, the interactive effect between habitat and season and an additive effect of spring migration timing, and the full three-way interaction; we again used the same methods for downstream statistical inference.

Vector surveillance

During our final two sampling years (2024 and 2025), we used Biogents-Sentinel 2 vector traps to monitor vector activity during our sampling period (January–May). We baited traps with BG-SweetScent mosquito attractant and dry ice as a source of carbon dioxide. We trapped vectors at three site replicates every week bird sampling occurred, either rotating trap sessions by habitat category (2024) or setting traps at three site replicates representing each habitat category on the same day (2025). Trap sessions lasted 24 hours, after which all insects were collected from the catch bag, euthanized by freezing, then stored in 96% ethanol. Insects were later sorted to

separate haemosporidian vector groups and identified to family (simuulid black flies), genus (ceratopogonid midges in genus *Culicoides*), or species (culicid mosquitoes).

RESULTS

Sampling distribution

We captured and collected blood samples from a total of 415 individual migratory sparrows across seven species (Figure 2.2): Dark-eyed Juncos (n = 217), Harris's Sparrows (n = 84), Fox Sparrows (n = 18), Lincoln's Sparrows (n = 41), Song Sparrows (n = 15), White-crowned Sparrows (n = 23), and White-throated Sparrows (n = 17). Captures were more numerous early in the sampling season when birds were organized in cohesive winter flocks, but species diversity was more evenly distributed in spring (Figure 2.2).

WNV positivity and seropositivity

No birds (0/415) harbored active WNV infections detectable using qRT-PCR. Of the 226 birds with sufficient plasma volumes for PRNT, 12 individuals were seropositive for WNV neutralizing antibodies at a 50% neutralization cutoff (Table 2.1). Seropositivity did not appear to be structured by habitat or season, but the number and distribution of positive samples were insufficient for statistical analysis. All three seropositive species in this study (i.e., Dark-eyed Juncos, Harris's Sparrows, and Lincoln's Sparrows) have previously been documented as hosts of WNV (Shelite *et al.*, 2008; George *et al.*, 2015).

Haemosporidian positivity

A total of 50% of birds were positive for haemosporidian infections using PCR (207/415, 95% CI: 45–55%). By parasite genus, total infection prevalence was 11% for *Plasmodium*, 12% for

Haemoproteus, and 28% for *Leucocytozoon.* Infections were distributed across 24 unique haemosporidian lineages previously recorded in the MalAvi database (Table 2.2).

Across all infection outcomes, additive-only models were more competitive than models including an interaction effect (Arnold 2010). The most competitive model for hfaemosporidian PCR positivity across all genera (*Plasmodium*, *Haemoproteus* and *Leucocytozoon*) included additive effects only of habitat type, ordinal date, and migration timing as well as body condition (w_i = 0.80, R^2_m = 0.03, R^2_c = 0.03; Table S2). Birds overwintering in rural habitats were more likely to host any haemosporidian infection than birds overwintering in suburban habitats (OR = 1.78, p = 0.05; Table S2) irrespective of sampling date. Additive-only models were also the best performers when positivity data were confined to a single haemosporidian genus, and fixed effects explained more variation in infection outcomes in single-genus models (Table S2). In the most competitive model solely considering infection with the genus *Plasmodium* (w_i = 0.83, R^2_m = 0.21, R^2_c = 0.35; Table S2), birds using suburban (OR = 7.86, p = 0.02) or rural (OR = 10.82, p = 0.01; Table 2) habitats were more likely to be infected than birds using urban habitats. In the most competitive model for *Haemoproteu*s positivity (w_i = 0.62, R^2_m = 0.22, R^2_c = 0.27; Table S2), birds using rural habitats were marginally more likely to be infected than birds using suburban habitats (OR = 3.48, p = 0.08). Ordinal sampling date was a strong predictor of *Haemoproteus* infection (OR per 1-day increase = 0.02, p < 0.01), and *Haemoproteus*-infected birds tended to be in poorer body condition (OR = -0.16, p = 0.04). In the most competitive model for *Leucocytozoon* positivity (w_i = 0.83, R^2_m = 0.02, R^2_c = 0.11; Table S2), ordinal date and land-use category did not predict infection.

Active haemosporidian infections

Active haemosporidian infections were defined as those with at least one gametocyte observed in the blood smear, which identifies a parasitized bird as a competent host harboring a transmissible infection (Valkiūnas *et al.*, 2009). Consistent with PCR positivity, the most competitive models for active infections with any genus (w_i = 0.77, R^2_m = 0.03, R^2_c = 0.03), for *Haemoproteus* only (w_i = 0.67, R^2_m = 0.22, R^2_c = 0.22), and for *Leucocytozoon* only (w_i = 0.80, R^2_m = 0.13, R^2_c = 0.13) included only additive effects of habitat type and ordinal date. No active infections were documented in our dataset for the genus *Plasmodium*. Habitat category and ordinal sampling date did not predict active infections in the global model considering infections with either haemosporidian genus or in the model including only *Leucocytozoon*. Ordinal sampling date significantly predicted active *Haemoproteus* infections in isolation (OR = 0.02 per 1-day increase, $p < 0.01$). Species categorized as early migrants were more likely to harbor active *Leucocytozoon* infections (OR = 2.79, $p < 0.01$), but active *Leucocytozoon* infection was not predicted by sampling date or habitat category.

Haemosporidian infection intensity

We only considered infection intensity separately for the genera *Haemoproteus* and *Leucocytozoon*, since no active infections were identified for the genus *Plasmodium,* and mean gametocyte counts in infected birds differed substantially between *Haemoproteus* ($\bar{x}$ = 56, SE = 2.6) and *Leucocytozoon* ($\bar{x}$ = 1, SE = 0.08). Additive-only models outperformed models including an interaction between habitat type and ordinal sampling date for both *Haemoproteus* (w_i = 0.90, R^2_m = 0.34, R^2_c = 0.34; Table S2) and *Leucocytozoon* infection intensity (w_i = 0.58; Table S2). Models for infection intensity excluded random effects to accommodate reduced sample sizes when only comparing birds infected with the same genus. *Haemoproteus* infection

intensity was not strongly predicted by ordinal sampling date or habitat category. *Leucocytozoon* infection intensity was marginally higher in rural habitats than in urban habitats (OR = 1.82, p = 0.07).

Haemosporidian within-host haplotype richness

Of 207 birds infected with haemosporidian parasites, 25 were coinfected with multiple lineages. The most competitive model for within-host haplotype richness again included only the additive effects of habitat type and ordinal sampling date (w_i = 0.84, R^2_m = 0.24, R^2_c = 0.35; Table S2). Neither habitat category nor sampling date were strong predictors of coinfection with multiple haemosporidian lineages (Figure 2.6).

Vector surveillance

We detected low occurrences of potential culicid vectors as early as January 29th and potential simuulid and ceratopogonid vectors as early as February 8th (Figure S2.2). Other than consistent but sparse winter and early spring observations suggestive of warm off-season temperatures causing some individuals to break diapause, vector observations were organized into discrete, local emergence events (i.e., capture events exceeding 10 individuals) in spring (Figure S2.2). A *Culicoides* biting midge emergence was noted in mid-March of 2024. Culicid mosquitoes began to emerge consistently between late April and the termination of sampling in May (Figure S2.2). Culicid emergences were dominated by *Aedes vexans* in April, followed by relatively even mixtures of *Aedes vexans, Culex tarsalis*, and *Anopheles punctipennis* in mid-May (Figure S2.2).

DISCUSSION

Understanding and mitigating the effects of accelerating anthropogenic change on migratory host populations will require bolstering our understanding of how land-use change interacts with infectious disease dynamics and how this relationship varies throughout the annual cycle. A significant rift in applying a full-annual cycle perspective to infectious disease research pertains to pathogens borne by arthropod vectors, which are almost exclusively studied during the breeding season when their vertebrate hosts are more frequently sampled (Marra *et al.*, 2015) and active transmission is occurring from arthropod vectors. However, pathogens such as haemosporidians and WNV could be maintained as chronic infections in overwintering host populations, with the potential to relapse in response to seasonal and/or anthropogenic stressors (Reisen *et al.*, 2006b; Dunn *et al.*, 2013; Becker *et al.*, 2020). Here, using overwintering sparrow communities, we explored the joint effects of seasonality (i.e., winter compared to spring) and land-use intensity on vector-borne infections with WNV and haemosporidians across the genera *Plasmodium*, *Haemoproteus* and *Leucocytozoon*. Our results suggest that both land-use and seasonality impact vector-borne infection outcomes during the stationary non-breeding season, but these relationships varied with the pathogen under study.

We did not find evidence for enzootic WNV activity in sparrows overwintering in central Oklahoma between mid-January and early May. Latency–reactivation cycles of chronic WNV infections in avian hosts and continued winter transmission at temperatures favoring mosquito activity and viral development (i.e. exceeding 14.3 C; (Reisen *et al.*, 2006a) have been proposed as mechanisms for the virus persisting between years (Reisen *et al.*, 2006b; Reisen and Wheeler, 2019). None of the overwintering sparrows in this study (n=415) tested positive for WNV RNA via qRT-PCR, which we identified as the most sensitive method for detecting infections across a

range of viremia. However, 5% of individuals yielding enough plasma to characterize the presence of WNV-neutralizing antibodies via PRNT were seropositive (Table 1). Birds exposed to WNV can retain detectable antibodies for months or years (Nemeth *et al.*, 2009), and the absence of PCR-detectable viremia or seasonal structure in seropositivity (Table 1) suggest seropositivity in our samples probably reflect WNV exposure during breeding or the fall migration period. New World sparrows are prone to short-term population declines after WNV outbreaks at their breeding grounds, followed by recovery (George *et al.*, 2015). Shared overwintering habitat in the Great Plains region being low-risk for continued WNV exposure may contribute to the relatively reliable recovery of passerellid sparrow populations after they first encounter the disease at their breeding grounds (George *et al.*, 2015).

Haemosporidian parasites of birds are a model system for studying eco-evolutionary relationships between hosts, vectors, and vector-borne pathogens, but studies comparing haemosporidian infection outcomes such as prevalence, intensity, and lineage diversity between urbanized and intact habitats have yielded variable results. Some research suggests urbanization increases haemosporidian infection outcomes, driven by factors such as negative environmental influences on host immunity and favorable microhabitats for key arthropod vectors (Bichet *et al.*, 2013; Caizergues *et al.*, 2024; Werner *et al.*, 2026). Alternatively, urban habitats might decrease haemosporidian infection outcomes by interrupting transmission from vectors with more specialized habitat requirements, decreasing host diversity (i.e., mitigating amplification effects by excluding competent host groups and/or suppressing transmission of host-specific lineages; Tamayo-Quintero *et al.*, 2023), or immune benefits from anthropogenic provisioning (Bonneaud *et al.*, 2009; Chasar *et al.*, 2009; Cornet *et al.*, 2014; Santiago-Alarcon *et al.*, 2020). However, other studies do not identify any link between haemosporidian infection and urbanization (Belo

et al., 2011; Bichet *et al.*, 2020). These discordant results highlight a context-dependent relationship between urbanization and haemosporidian infection in wild birds, and our findings support that important ecological contexts include haemosporidian genus, the position of modified habitats on the urban gradient (Caizergues *et al.*, 2024; Reid *et al.*, 2024), and seasonality within even a single stage of the annual cycle (Dominoni *et al.*, 2026).

Seasonality and habitat land-use category better explained infection outcomes when models were restricted to a single haemosporidian genus (Table S2), underscoring ecological differences in how *Plasmodium*, *Haemoproteus*, and *Leucocytozoon* interact with their hosts and environment. For the genus *Haemoproteus*, PCR-detected infections were more common in rural habitats than in suburban habitats. This is consistent with previous research on the relationship between *Haemoproteus* infection and urbanization and may partially be because *Haemoproteus* contains a high proportion of host-specialist lineages that fare poorly in homogenized urban bird communities (Chasar *et al.*, 2009; Amaya-Mejia *et al.*, 2025). *Haemoproteus* was also the only genus with a strong seasonal component to infection, with prevalence increasing exponentially with ordinal sampling date (i.e., closer to spring migration; Figures 2.3c and 2.4b). This pronounced spring peak in infection prevalence indicates that migratory relapse and/or active vector transmission mediate *Haemoproteus* infection in sparrows overwintering in Oklahoma before spring migration (Becker *et al.*, 2020; Binning *et al.*, 2022). We observed a similar rate of increase in occurrence of both chronic and active infections (Figures 2.3c and 2.4b) as well as a *Culicoides* biting midge emergence event in mid-March (Figure S2.2), suggesting the observed increase in prevalence was more likely to be caused by new infections from arthropod vectors than by relapsing chronic infections. In contrast, *Haemoproteus* infection intensity was not related to season or habitat land-use category (Figure 2.5). The lack of seasonal structure in

infection intensity may be due to a non-linear relationship that was not captured by our analyses, given Haemoproteus infection intensity and ceratopotogonid midge activity seemed to peak in early spring (e.g., during the migratory period for species categorized as early migrants). This pattern may have confounded results given that we could not account for differences between early- and late-migrating host species in the reduced model (Table S2.2, Figure S2.2).

Leucocytozoon was the most common haemosporidian genus infecting the bird communities in our study (28% infection prevalence), which is unsurprising given the high prevalence of this group in previous studies of New World sparrows (Deviche *et al.*, 2001; Martínez-Renau *et al.*, 2022; Grieves *et al.*, 2023). Habitat category and ordinal sampling date did not predict the combined prevalence of chronic and active *Leucocytozoon* infections using PCR. Active *Leucocytozoon* infections were common in sparrows overwintering in Oklahoma, especially in early winter, and tended to be more intense in birds using rural habitats than birds using suburban habitats (Figures 2.3d, 2.4c). Active *Leucocytozoon* infections during winter months have been documented in previous studies (Hauptmanová *et al.*, 2002; Barnard *et al.*, 2010) and may be attributed to stress-related relapse events or the year-round presence of black flies. It seems most plausible that active *Leucocytozoon* infections here represent carry-over infections transmitted during the breeding season, leaving enduring but sparse numbers of circulating gametocytes, a trait unique to genus *Leucocytozoon* (Valkiunas, 2004). This is supported by our inability to detect black fly emergence events from vector traps throughout the season (Figure S2.2) and by the previous recovery of the two most common *Leucocytozoon* lineages in this study, ZOLEU02 and CNEORN01 (Table 2.2), from birds at Canadian and Alaskan breeding grounds used by all seven of our focal species (Galen *et al.*, 2018; Martínez-Renau *et al.*, 2022). Host species categorized as early migrants (Figure S2.1) were also more

likely to harbor active *Leucocytozoon* infections, potentially reflecting phenological differences in overlap with black fly vector and/or haemosporidian parasite communities at the breeding grounds.

Consistent with the results for genus *Leucocytozoon*, we found birds had a lower risk of being infected with parasites from the genus *Plasmodium* when they overwintered in urban habitats than when they overwintered in rural habitats. The only *Plasmodium* infections we observed in sparrows were chronic, suggesting that like *Leucocytozoon*, active *Plasmodium* transmission was unlikely to be occurring while birds were overwintering. One possible explanation for this pattern is that the varying overwintering habitat conditions experienced by our focal species between their arrival in Oklahoma in October and the start of our sampling in January interacted with *Plasmodium* and *Leucocytozoon* infections birds contracted earlier in their annual cycle. Supplemental nutrition may suppress haemosporidian infections by allowing birds to better invest in immune defense (Cornet *et al.*, 2014; Becker *et al.*, 2015), and more urbanized wintering habitats are likely to contain abundant and spatiotemporally predictable sources of anthropogenic provisioning like bird feeders. The potential costs of using urbanized wintering habitats could be offset by nutritional benefits for species like granivorous New World sparrows that can exploit anthropogenic resources, helping birds clear infections they obtained during the breeding season, but this is conjecture warranting further study.

Seasonal arthropod vector activity across our sites largely reflected the infection patterns we observed in bird communities. Vector presence in BG-Sentinel mosquito traps during winter and early spring was limited to few, if any, individuals breaking diapause (Figure S2.2). As previously discussed, we detected a *Culicoides* biting midge emergence event in March (Figure S2) consistent with rising *Haemoproteus* prevalence in mid-spring (Figures 2.3c and 2.4b).

Culicid species began emerging between mid-April and the termination of vector surveillance in mid-May. Early culicid emergences were dominated by *Ae. vexans,* a mammophilic species that may serve as a bridge vector to mammalian hosts in WNV transmission but seldom feeds on birds and is not a primary vector of haemosporidians (Molaei and Andreadis, 2006; Bobeva *et al.*, 2026). On May 9th and 14th, we detected groups of *Anopheles punctipennis* and *Culex tarsalis* in vector traps in addition to *Ae. vexans* (Figure S2.2). *An. punctipennis* is a vector of mammalian *Plasmodium* in the Great Plains region, but has not been implicated in the transmission of WNV or avian haemosporidians (Noden *et al.*, 2021). In contrast, *C. tarsalis* is a vector of both WNV and avian *Plasmodium* (Noden *et al.*, 2021; Maichak *et al.*, 2022). Our failure to detect *C. tarsalis* or other potential vectors before New World sparrow populations had largely migrated from Oklahoma in mid-spring (Figure S2.1) supports that the absence of WNV or active *Plasmodium* infections in spring was caused by temporal separation of migratory bird and vector abundances (Galen *et al.*, 2024). We note the important limitation that the spring biting midge and mosquito emergences we observed were small and highly localized across space and time. Our weekly vector sampling regime therefore made it likely that emergence events and diversity went undetected and precluded the identification of any fine-scale temporal patterns or effects of habitat land-use category on vector activity.

The stationary non-breeding season is an understudied but critical part of the annual cycle (Marra *et al.*, 2015), which allows temperate migrants to escape harsh seasonal conditions at their breeding grounds and accumulate resources that can both predict future fitness and offset negative carryover effects from previous stages (Senner *et al.*, 2014; Briedis *et al.*, 2018). We find that overwintering can also be a surprisingly impactful period for vector-borne disease, and infection outcomes vary both by the pathogen under study and intra-seasonal context (i.e.,

anthropogenic land-use intensity, proximity to spring migration). We did not find support for the hypothesis that the coupling of urban and migratory stressors before spring migration amplify vector-borne infection outcomes; in contrast, birds wintering in rural habitats had the highest haemosporidian infection prevalence, and we could not reconcile this pattern with differences in vector activity. Our results indicate that both acute and chronic haemosporidian infections affect overwintering migrants and motivate future study on the effects of vector-borne pathogens on migratory hosts outside the breeding season.

TABLES

Table 2.1. WNV seropositivity across 249 plasma samples tested by PRNT stratified by host species, overwintering habitat category, and season. Samples were considered seropositive if they exceeded 50% antibody neutralization at a final plasma dilution of 1:20.

Host species	Total seroprevalence	Rural	Suburban	Urban	Winter	Spring
Dark-eyed Junco	0.08 (9/104)	0.10 (3/29)	0.17 (6/36)	0 (0/39)	0.09 (7/75)	0.07 (2/29)
Fox Sparrow	0 (0/10)	0/6 (0/6)	0 (0/4)	N/A (0/0)	0 (0/8)	0 (0/2)
Harris's Sparrow	0.03 (2/65)	0.04 (1/23)	0.03 (1/30)	0 (0/12)	0.02 (1/45)	0.05 (1/20)
Lincoln's Sparrow	0.08 (1/13)	0 (0/5)	0.25 (1/4)	0 (0/4)	0 (0/2)	0.09 (1/11)
White-crowned Sparrow	0 (0/16)	0 (0/8)	0 (0/3)	0 (0/5)	0 (0/2)	0 (0/14)
Song Sparrow	0 (0/8)	0 (0/5)	0 (0/3)	N/A (0/0)	0 (0/5)	0 (0/3)
White-throated Sparrow	0 (0/10)	0 (0/3)	0 (0/6)	0 (0/1)	0 (0/5)	0 (0/5)
All	0.05 (12/226)	0.05 (4/79)	0.09 (8/86)	0 (0/61)	0.05 (8/142)	0.05 (4/84)

TABLES

Table 2.2. Summary of haemosporidian lineages assigned using the MalAvi database, morphospecies, number of birds infected, and list of host species (DEJU = Dark-eyed Junco, SOSP = Song Sparrow), FOSP = Fox Sparrow, HASP = Harris's Sparrow, LISP = Lincoln's Sparrow, WCSP = White-crowned Sparrow, WTSP = White-throated Sparrow).

MalAvi lineage	Morphospecies	# observations	Host species
BT7	*Plasmodium* sp.	25	DEJU, FOSP, HASP, LISP, WTSP
GEOTRI09	*Plasmodium* sp.	4	DEJU, SOSP, WCSP
LAIRI01	*Plasmodium* sp.	2	DEJU, LISP
PADOM11	*Plasmodium* sp.	5	DEJU, FOSP, HASP, SOSP, WTSP
RWB01	*Plasmodium* sp.	4	LISP, SOSP, WTSP
SEIAUR01	*Plasmodium cathemerium*	7	DEJU, FOSP, HASP
SETCOR03	*Plasmodium* sp.	1	DEJU
DUNNO01	*Haemoproteus* sp.	4	LISP, WCSP
GYMSAL01	*Haemoproteus* sp.	23	DEJU, HASP, WCSP, WTSP
JUHYE02	*Haemoproteus* sp.	3	DEJU
MELLIN01	*Haemoproteus* sp.	2	LISP
PIPMAC03	*Haemoproteus* sp.	6	DEJU, HASP, WCSP
SPIARB01	*Haemoproteus* sp.	10	DEJU, HASP, WCSP
ZOCAP08	*Haemoproteus* sp.	1	DEJU
ACAFLA03	*Leucocytozoon* sp.	4	DEJU, HASP
CARCAN01	*Leucocytozoon* sp.	1	DEJU
CB1	*Leucocytozoon majoris*	6	DEJU, HASP
CNEORN01	*Leucocytozoon* sp.	42	DEJU, FOSP, HASP, LISP
DENCOR05	*Leucocytozoon* sp.	1	WCSP
DUMCAR01	*Leucocytozoon* sp.	2	SOSP, WTSP
SETSTR01	*Leucocytozoon* sp.	7	FOSP, HASP
SPIPAS07	*Leucocytozoon* sp.	2	DEJU
ZOLEU02	*Leucocytozoon fringillinarum*	45	DEJU, FOSP, HASP, LISP, SOSP, WCSP
ZOLEU06	*Leucocytozoon* sp.	1	HASP

FIGURES

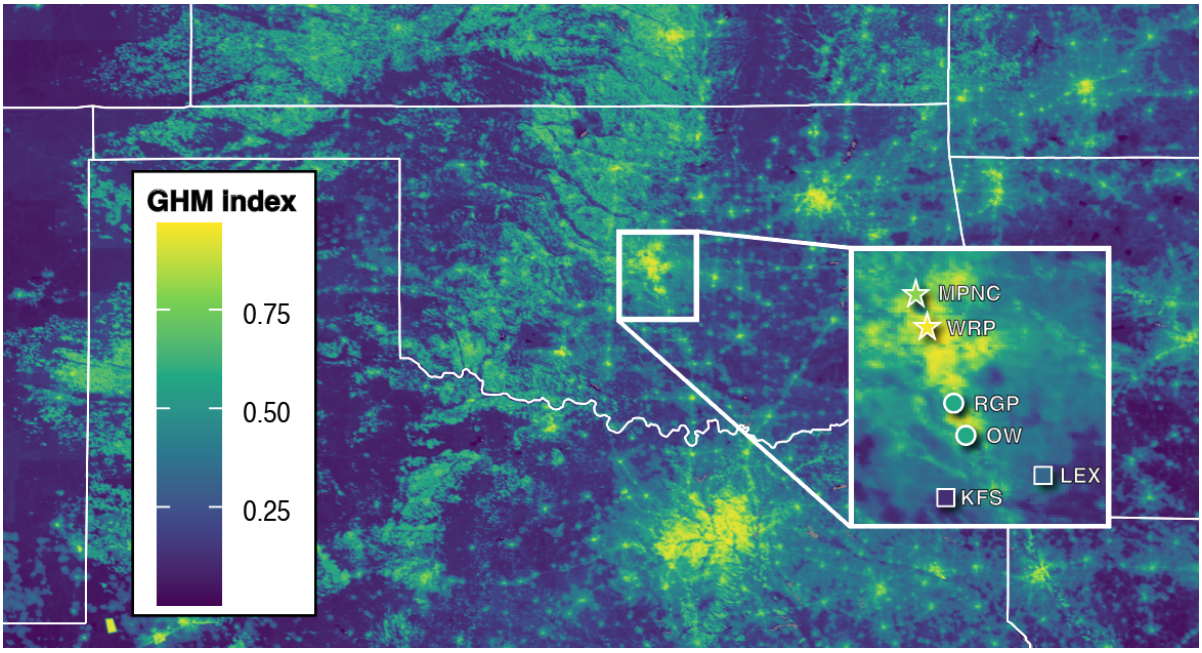


Figure 2.1. Global Human Modification Index (GHM; (Kennedy *et al.*, 2020) map depicting the study's six sampling sites in central Oklahoma (MPNC = Martin Park Nature Center, WRP = Will Rogers Park, RGP = Ruby Grant Park, OW = Oliver's Woods, KFS = Kessler Atmospheric and Ecological Field Station, LEX = Lexington Wildlife Management Area). Icons denote habitat land-use categories (star = urban, circle = suburban, square = rural) defined using the GHM within the 30-hectare estimated wintering home range of Dark-eyed Juncos as a representative target species.

FIGURES

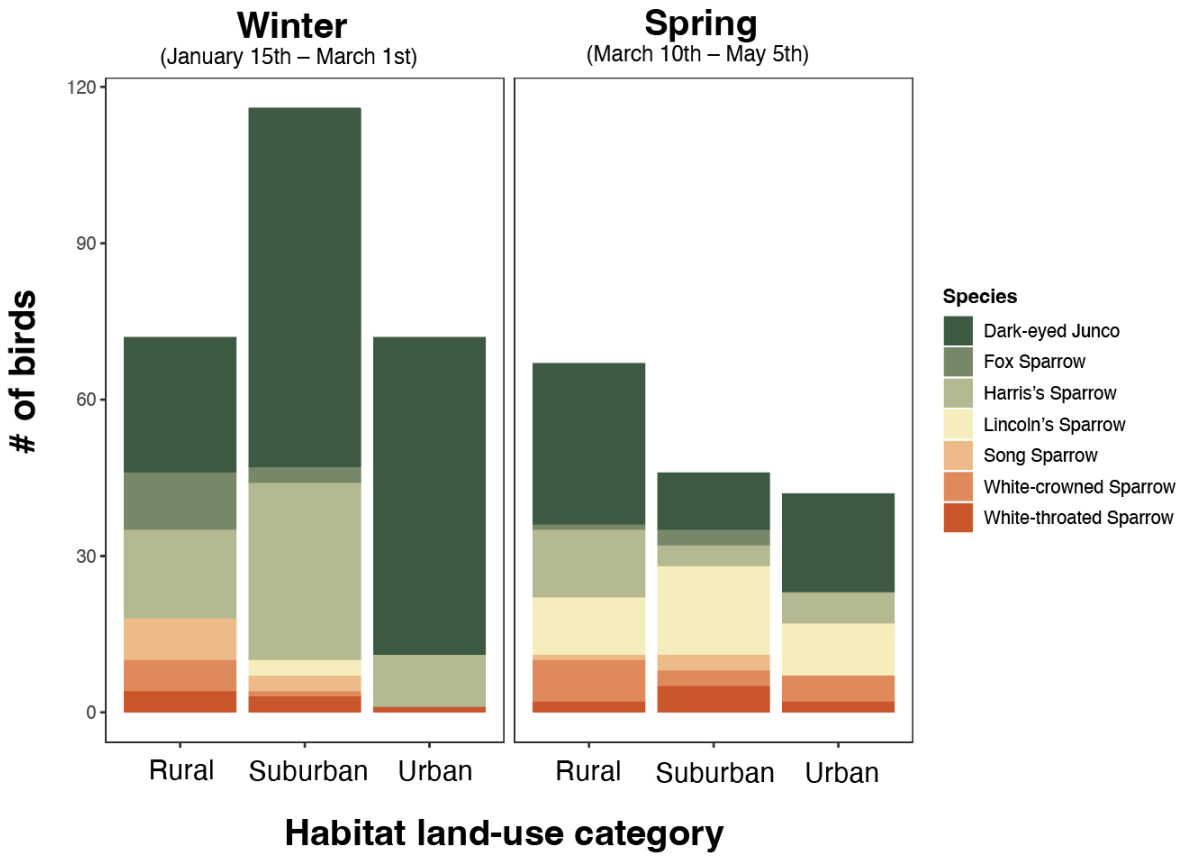


Figure 2.2. Sampling distribution of $n = 415$ overwintering migratory sparrows captured across 6 site replicates in central Oklahoma, organized by species, overwintering habitat category, and season.

FIGURES

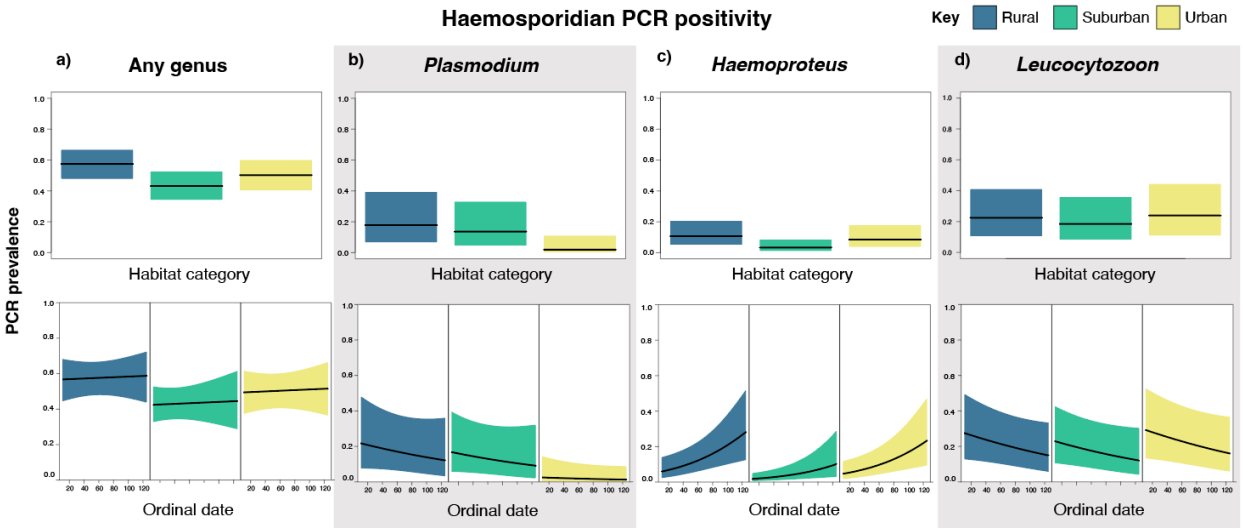


Figure 2.3. Conditional plots of the estimated effects of habitat land-use category (top; rural, suburban, or urban) and joint habitat category and ordinal sampling date (bottom) on haemosporidian PCR positivity across (a) all genera, (b) *Plasmodium* only, (c) *Haemoproteus* only, and (d) *Leucocytozoon* only. 95% confidence intervals are shaded by habitat category. Plots were generated from the most competitive generalized linear mixed effect (GLMM) model using the package *visreg*.

FIGURES

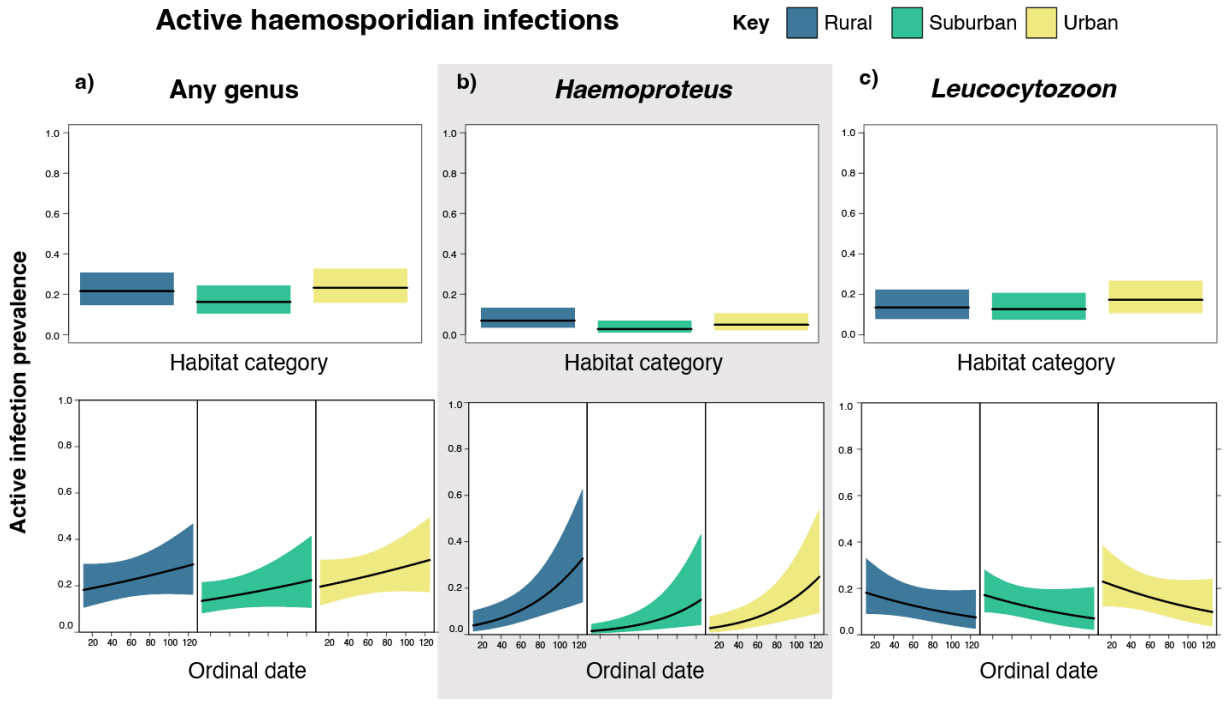


Figure 2.4. Conditional plots of the estimated effects of habitat land-use category (top; rural, suburban, or urban) and joint habitat category and ordinal sampling date (bottom) on active haemosporidian infection positivity across (a) all genera, (b) *Haemoproteus* only, and (c) *Leucocytozoon* only. Active infections were defined as those with at least one gametocyte identified in the blood smear. 95% confidence intervals are shaded by habitat category. Plots were generated from the most competitive GLMM (Table 2.2) using the package *visreg*.

FIGURES

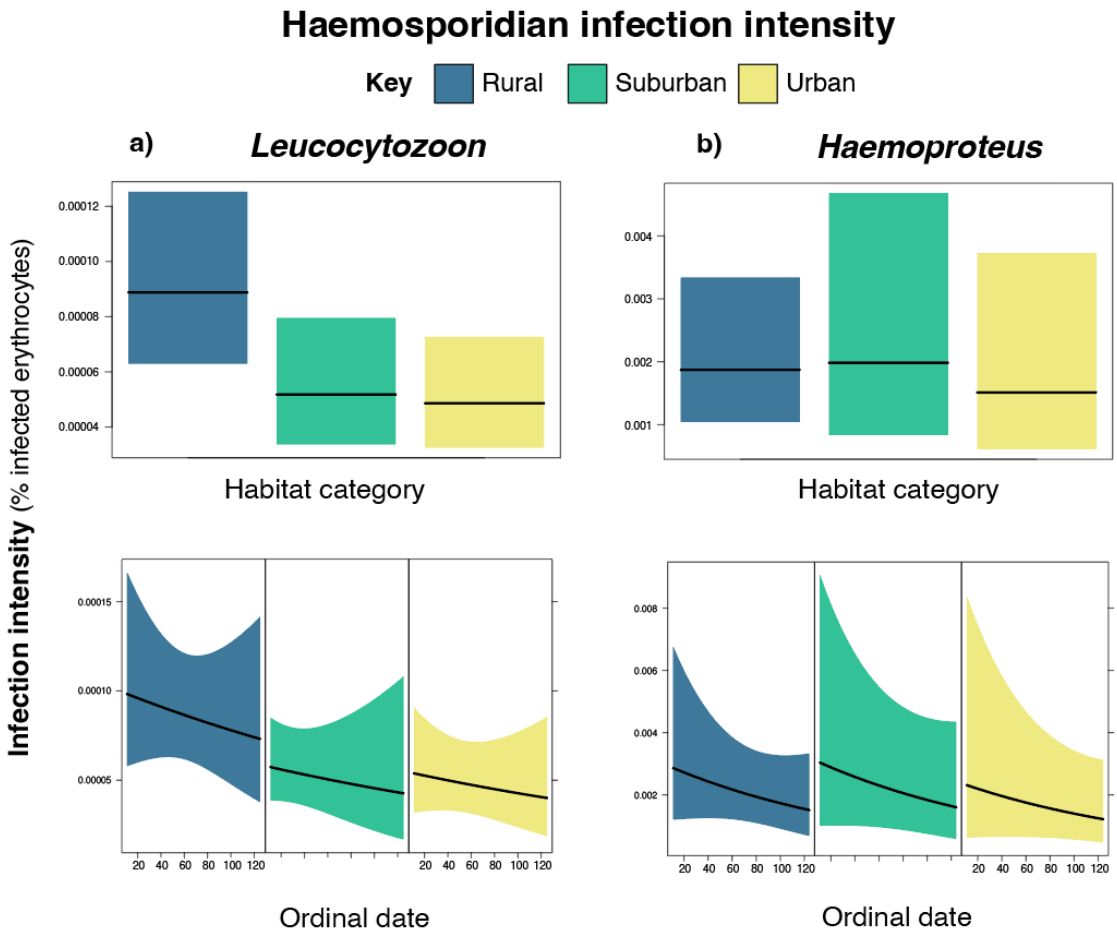


Figure 2.5. Conditional plots of the estimated effects of habitat land-use category (top; rural, suburban, or urban) and joint habitat category and ordinal sampling date (bottom) on haemosporidian infection intensity for genera (a) *Haemoproteus* and (b) *Leucocytozoon*. Infection intensity was defined as the proportion of infected erythrocytes across 100 fields of view (~43,000 erythrocytes). 95% confidence intervals are shaded by habitat category. Plots were generated from the most competitive GLM (Table S2) using the package *visreg*.

FIGURES

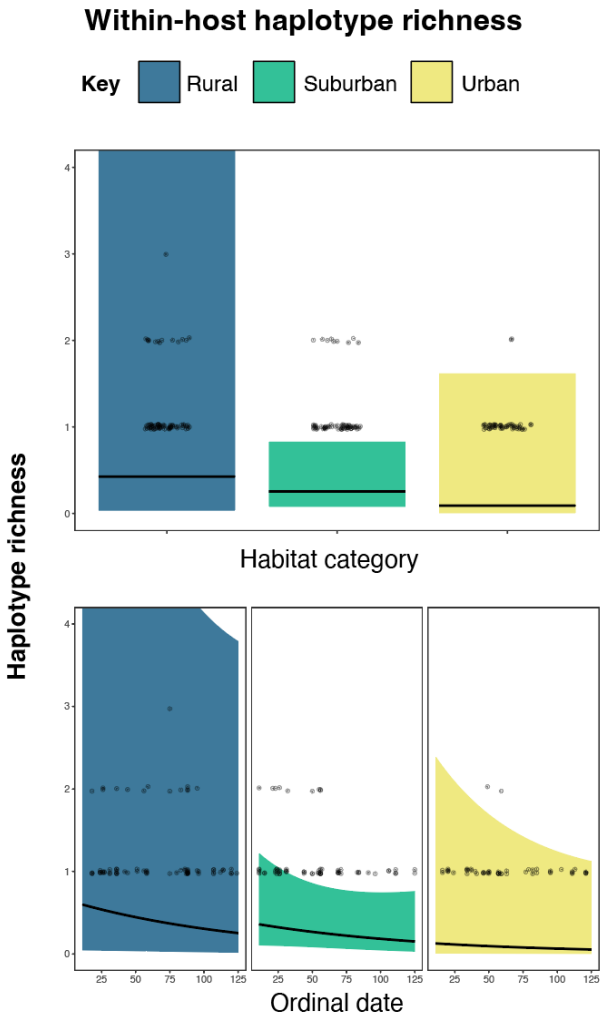


Figure 2.6. Conditional plots of the estimated effects of habitat land-use category (top; rural, suburban, or urban) and joint habitat category and ordinal sampling date (bottom) on within-host haplotype richness (i.e., coinfections with 1-3 haemosporidian lineages).

SUPPLEMENTAL TABLES AND FIGURES

Table S2.1. Primers used for comparing West Nile virus detection via qRT-PCR across a range of viremia.

Assay	Target	Primer	Sequence	Amplicon size
qRT-PCR	env	Forward	TCAGCGATCTCTCCACCAAAG	70 bp
		Reverse	GGGTCAGCACGTTTGTCATTG	
		Probe	FAM-TGCCCGACCATGGGAGAAGCTC	
PCR	sfRNA	Forward	AATGTCAGACCACGCTACGG	148 bp
		Reverse	ACCTCTAGTCCTTTCGCCCT	
	NS5	Forward	TGAGGAGCGCGAGGCACAT	177 bp
		Reverse	GTCTTCATTGAGAAAACCCAGAGC	

SUPPLEMENTAL TABLES AND FIGURES

Table S2.2. Model comparisons for haemosporidian PCR positivity, active infection positivity, infection intensity, and within-host haplotype richness. Model fit was assessed using AICc, with the best-performing model represented by the lowest AICc. Analyses for *Haemoproteus* and *Leucocytozoon* infection intensity did not include random effects to accommodate the much smaller sample size of infected birds.

Haemosporidian infection outcome	**Model fixed effects**	**k**	**ΔAICc**	**w_i**	**R^2_m**	**R^2_c**
PCR positivity, any	habitat category + ordinal date + migration timing + body condition	9	0	0.80	0.03	0.03
	habitat category + ordinal date * migration timing + body condition	11	2.92	0.19	0.03	0.03
	habitat category * ordinal date * migration timing + body condition	16	8.41	0.01	0.05	0.05
PCR positivity, genus *Plasmodium*	habitat category + ordinal date + migration timing + body condition	9	0	0.83	0.21	0.35
	habitat category * ordinal date + migration timing + body condition	11	3.11	0.17	0.25	0.37
PCR positivity, genus *Haemoproteus*	habitat category + ordinal date + migration timing + body condition	9	0	0.62	0.22	0.27
	habitat category * ordinal date + migration timing + body condition	11	0.95	0.38	0.23	0.25
	habitat category * ordinal date * migration timing + body condition	16	10.77	0	0.24	0.26
PCR positivity, genus *Leucocytozoon*	habitat category + ordinal date + migration timing + body condition	9	0	0.83	0.02	0.11
	habitat category * ordinal date + migration timing + body condition	11	3.41	0.15	0.02	0.11
	habitat category * ordinal date * migration timing + body condition	16	8.09	0.02	0.04	0.14

SUPPLEMENTAL TABLES AND FIGURES

Table S2.2 (*continued*).

Haemosporidian infection outcome	Model fixed effects	k	ΔAICc	w_i	R^2_m	R^2_c
Active infection, any genus	habitat category + ordinal date + migration timing + body condition	9	0	0.77	0.03	0.03
	habitat category * ordinal date + migration timing + body condition	11	2.67	0.20	0.04	0.05
	habitat category * ordinal date * migration timing + body condition	16	6.66	0.03	0.08	0.15
Active infection, genus *Haemoproteus*	habitat category + ordinal date + migration timing + body condition	9	0	0.67	0.22	0.22
	habitat category * ordinal date + migration timing + body condition	11	1.43	0.33	0.28	0.28
	habitat category * ordinal date * migration timing + body condition	16	8.93	0	0.34	0.34
Active infection, genus *Leucocytozoon*	habitat category + ordinal date + migration timing + body condition	9	0	0.80	0.13	0.13
	habitat category * ordinal date + migration timing + body condition	11	2.99	0.18	0.14	0.14
	habitat category * ordinal date * migration timing + body condition	16	7.83	0.02	0.20	0.20
Infection intensity, genus *Haemoproteus*	habitat category + ordinal date + body condition	7	0	0.84	N/A	N/A
	habitat category * ordinal date + body condition	9	3.34	0.16	N/A	N/A
Infection intensity, genus *Leucocytozoon*	habitat category + ordinal date + body condition	7	0	0.57	N/A	N/A
	habitat category * ordinal date + body condition	9	0.57	0.43	N/A	N/A
Within-host haplotype richness	habitat category + ordinal date + body condition	8	0	0.84	0.24	0.35
	habitat category * ordinal date + body condition	10	3.24	0.16	0.27	0.38

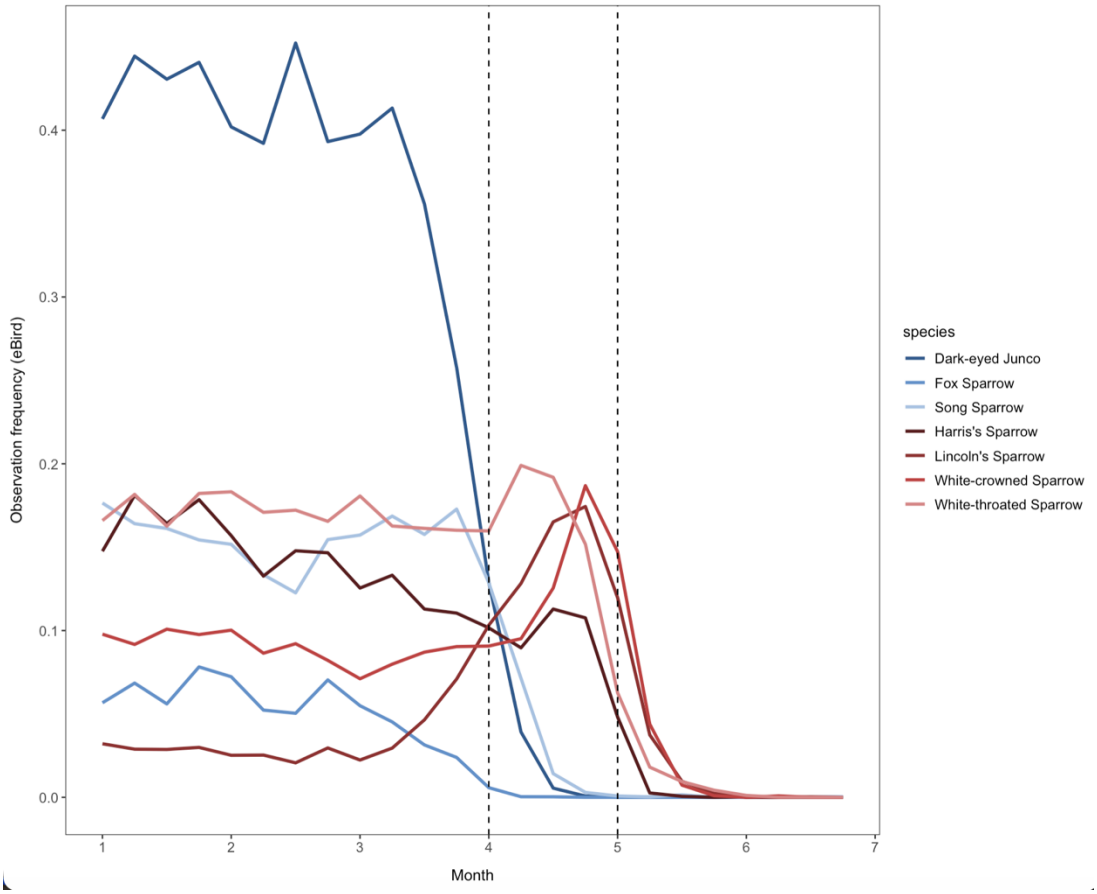


Figure S2.1. Migratory phenology of "early-migrating" (Dark-eyed Juncos, Fox Sparrows, and song Sparrows) and "late-migrating" (Harris's Sparrows, Lincoln's Sparrows, White-crowned Sparrows, and White-throated Sparrows) in Oklahoma, visualized using eBird weekly bar charts for species observations in Oklahoma between 2021 and 2025.

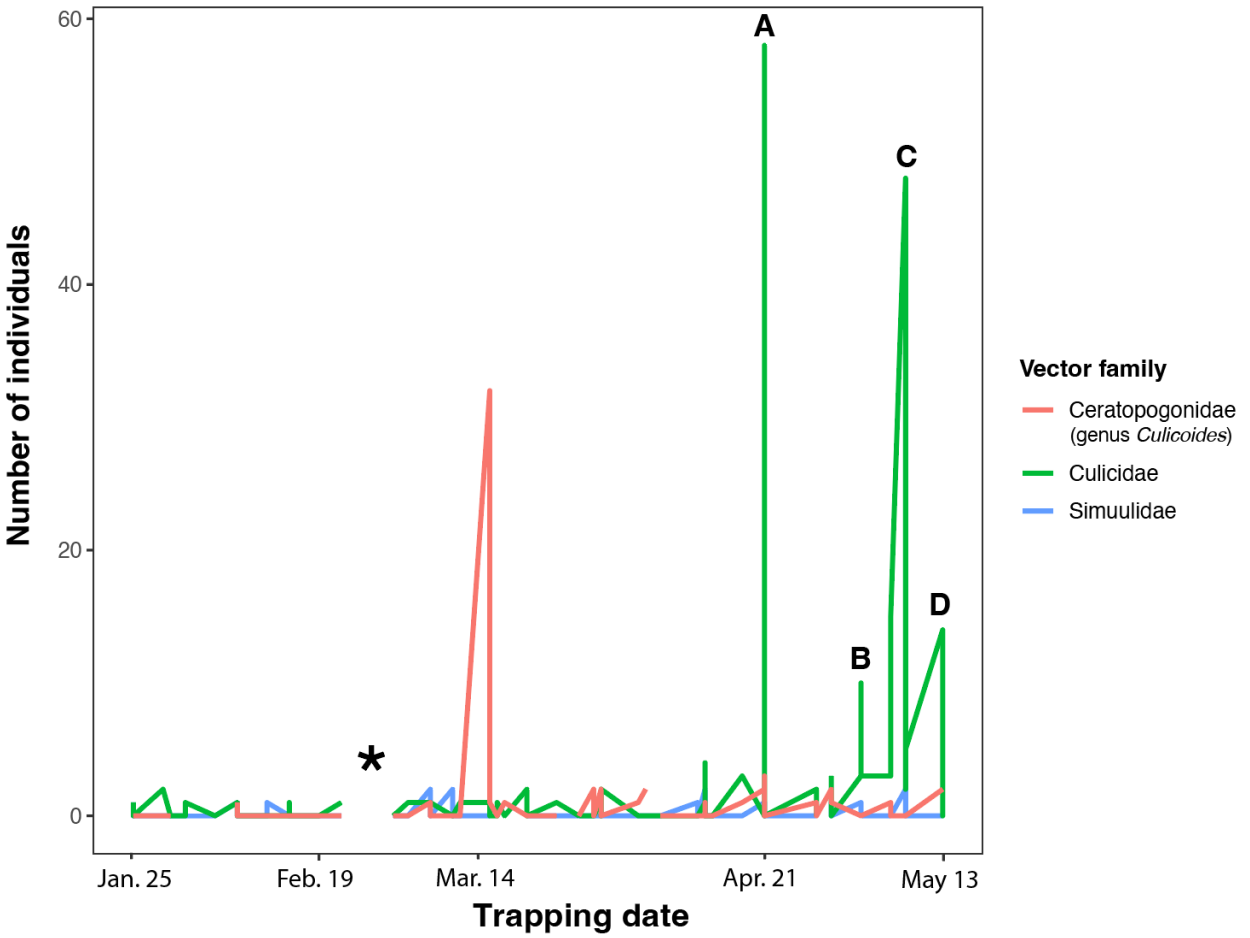


Figure S2.1. Combined results of vector surveillance during 2024 and 2025 using BG-Sentinel vector traps. Culicid species identified from trapping sessions yielding ≥ 10 individuals are as follows, in order of abundance for sessions containing multiple species: a) *Aedes vexans*, b) *Aedes vexans*, c) *Culex tarsalis*, *Aedes vexans*, and *Anopheles punctipennis*, and d) *Culex tarsalis*, *Anopheles punctipennis*, and *Aedes vexans*. * Pause between winter and spring sampling sessions

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CHAPTER 3

Migration but not urban tolerance influences cophylogenetic congruence between birds and their haemosporidian parasites

INTRODUCTION

Urbanization is among the most permanent and transformative types of anthropogenic land use (McKinney, 2009). The loss, degradation, and conversion of natural habitats with urbanization poses a mounting threat to the planet's biodiversity, especially as the pace of urban expansion continues to accelerate to accommodate human population growth (Angel *et al.*, 2011). However, urban expansion does not affect all species equally, nor does it affect all species negatively. Wildlife responses to urbanization rely on a suite of species-specific ecological, behavioral, physiological, and life history characteristics, which can sometimes be linked to shared evolutionary history (Rodewald and Gehrt, 2014; Iglesias-Carrasco *et al.*, 2022; Betke *et al.*, 2023). In other words, although the net effect of urbanization on biodiversity is negative, any given species occupies a continuum of urban tolerance based on how well its functional traits suit it to urban life (the urban tolerance hypothesis; (Sol *et al.*, 2014). At one end of this continuum, "urban avoiders" are sensitive to anthropogenic disturbance and tend to be excluded from urban environments (Blair, 1996). At the other extreme, species termed "urban exploiters", "urban adapters", or "synanthropes" are highly tolerant of modified habitats and can effectively use, occupy, and achieve high population densities in urban environments (Blair, 1996); (Francis and Chadwick, 2012); (Santini *et al.*, 2019).

The ecological traits typifying synanthropes are relatively well-characterized in wild birds. Globally, urban-tolerant bird species tend to be smaller-bodied, gregarious, more

dispersal-prone, and have broader niche breadths in terms of both dietary and habitat generalism (Bonier *et al.*, 2007; Møller, 2009; Neate-Clegg *et al.*, 2023). The contribution of these functional traits to urban tolerance can vary with geography (Neate-Clegg *et al.*, 2023) but is relatively consistent. While urban-tolerant mammals have adopted diverse and taxonomically stratified strategies for urban life (Santini *et al.*, 2019; Betke *et al.*, 2023), urban bird communities are generally defined by this set of "winning" traits irrespective of phylogeny (Evans *et al.*, 2011), (Santini *et al.*, 2019). As the species-specific traits associated with urban tolerance filter the rapidly homogenizing bird diversity present in anthropogenic habitats, these traits should become increasingly represented in urban bird communities (Luck and Smallbone, 2011).

Depauperate diversity in urban communities and increased densities of urban-adapted species can have consequences for infectious diseases. Urban-adapted species often harbor greater parasite richness than species unassociated with urban landscapes and can serve as reservoir hosts, increasing infection prevalence in their communities (Marm Kilpatrick *et al.*, 2006; Albery *et al.*, 2022; Ecke *et al.*, 2022; Wei *et al.*, 2026). While most comparative work to date has focused on how urban tolerance shapes variation in parasite diversity and abundance, this characteristic could also favor parasites with certain traits. For example, urbanization may favor transmission of pathogens spread through direct contact owing to density-dependent transmission and close contact around clumped anthropogenic resources (Murray *et al.*, 2019). Beyond transmission mode, an equally fundamental feature of parasites is their host specificity, a measure of ecological specialization that can reflect both the number and phylogenetic breadth of hosts a parasite can exploit (Poulin, 2007). Parasites with high host specificity are associated with a single or few closely related hosts (i.e., "host specialists"), whereas parasites with low

specificity may use numerous, phylogenetically distant hosts (i.e., "host generalists"). Host specialists are often assumed to have diversified via cospeciation, resulting in evolutionary histories that mirror that of their hosts'. In contrast, host generalists are expected to have their co-evolutionary congruence disrupted by relatively frequent host-switching events.

Host specificity and emergent macro-evolutionary patterns (e.g., evolutionary histories shaped predominantly by host switching or by cospeciation) are influenced by both host and vector ecology, particularly in parasites that are not free-living (Huyse et al. 2005). For example, solitary social systems in hosts can reinforce high specificity and cospeciation with their parasites (Hafner *et al.*, 1994), while host migratory propensity or dispersal ability can instead lead to host-switching opportunities and lower co-evolutionary congruence (Jenkins *et al.*, 2012); (Boyd *et al.*, 2022); but see (de Angeli Dutra *et al.*, 2022). However, whether co-phylogenetic congruence and host switching of parasites is modified by the unique ecological traits associated with urban tolerance remains poorly understood.

Here, we test the contribution of urban tolerance in avian hosts to cophylogenetic relationships with their haemosporidian parasites. Haemosporidian parasites of birds (in the genera *Plasmodium*, *Haemoproteus*, and *Leucocytozoon*) are cosmopolitan, intra-erythrocytic protozoa vectored by mosquitoes, biting midges, hippoboscid flies, and black flies. Haemosporidian infections are highly prevalent in wild birds and are often associated with sparse and sub-lethal effects on their hosts, such as decreases in body condition and reproductive success (Valkiūnas 2005). However, their pathogenicity is both lineage- and context-dependent, and haemosporidian infections have been implicated in the decline and extirpation of multiple bird populations (Woodworth *et al.*, 2005; Dadam *et al.*, 2019). Avian haemosporidia demonstrate variable degrees of host specificity, ranging from host specialists recovered

consistently from a single species to generalist lineages associated with a global range of hosts (Ricklefs *et al.*, 2005). The prevalence, diversity, and varying host specialization strategies used by haemosporidians underpin their status as a model system for studying host–parasite interactions. For example, bird–haemosporidian associations are a valuable empirical system for testing hypotheses at the interface of host migration and parasitism (Emmenegger *et al.*, 2018; de Angeli Dutra *et al.*, 2021a). Avian haemosporidia are also especially useful for testing hypotheses related to the contributions of host–parasite links to overall cophylogenetic congruence, because they have patterns of diversification driven both by host switching and cospeciation (Ricklefs *et al.*, 2004; Santiago-Alarcon *et al.*, 2014).

We predict the broader niche breadth and higher dispersal ability of urban-tolerant bird species could expose haemosporidian parasites of these hosts to greater environmental heterogeneity, favoring selection for host generalism and diversification via host-switching events (Kassen 2002). Alternatively, bird species more prone to using disturbed habitats may expose their parasites to less avian host diversity, which could curb selection for host generalism (Moens and Pérez-Tris, 2016). Migration strategy may also influence cophylogenetic congruence either independently or in tandem with urban tolerance, given the widespread taxonomic and geographic distribution of migratory behavior among birds (Dufour *et al.*, 2020; Tobias *et al.*, 2022). A migratory host could promote host-switching events by increasing encounters with parasites and periodically suppressing host immunity (Arriero and Møller, 2008; Eikenaar and Hegemann, 2016); alternatively, migration could instead favor co-evolution of parasites to better exploit a host with seasonally variable internal and external environments (Clayton *et al.*, 2003). An urban-tolerant, generalist host species may subject its parasites to similar pressures at a smaller ecological scale, which could result in convergent cophylogenetic patterns between

parasites and birds that are resident but urban-tolerant and birds that are migratory but urban-intolerant. To test these predictions, we employed a global database of associations between haemosporidian lineages and avian host species and then used the Procrustean Approach to Cophylogeny (PACo) and phylogenetic generalized linear mixed models (PGLMMs) to test if host–parasite links associated with urban-adapted and migratory bird species have lower contributions to overall cophylogenetic congruence.

METHODS

Host–parasite data

We downloaded all available haemosporidian lineages from the MalAvi database (accessed on 2/2/2025), a comprehensive, standardized, and dynamically updated record of haemosporidian cytochrome *b* sequences from avian hosts (Bensch *et al.*, 2009). We conservatively pruned the dataset to include only hosts identified to species and to those host–parasite links recorded at least twice to reduce including erroneous associations while attempting to preserve signal from rare and/or specialist haemosporidian lineages. We also removed any lineages from MalAvi that were substantially shorter than the standard 479 bp cytochrome *b* fragment used to characterize haemosporidian diversity (i.e., retained haplotypes ≥400 bp). The trimmed MalAvi alignment included a total 1,036 haemosporidian lineages (298 in the genus *Plasmodium*, 480 in the genus *Haemoproteus*, and 258 in the genus *Leucocytozoon*). The resulting dataset comprised 2,296 associations between birds and haemosporidian parasites.

Host and parasite phylogeny

We standardized host species names in MalAvi against the BirdTree taxonomy and then used BirdTree to produce a consensus phylogeny for passerine hosts using 2,000 randomly sampled trees generated from the Hackett backbone (Jetz *et al.*, 2012). We removed bird species not present in the MalAvi database, resulting in a total 743 host species spanning 94 avian families. We constructed a maximum likelihood phylogeny of haemosporidian parasite lineages with RAxML-NG (Kozlov *et al.*, 2019) as implemented in raxmlGUI 2.0 (Edler *et al.*, 2021), using 100 bootstrap replicates and a GTR+I+G substitution model (Abadi *et al.*, 2019). This number of bootstrap replicates is sufficient for maximum likelihood phylogenetics (Pattengale *et al.*, 2010).

Host species traits

We next assigned habitat breadth, urban occurrence, disturbed habitat occurrence, an urban tolerance index (UTI), and an avoider/exploiter metric to each host species present in the MalAvi database. We used the BirdBase dataset to assign habitat breadth, defined as the sum of International Union for Conservation of Nature (IUCN) habitat classification types (e.g., grassland, wetland, artificial) used by a given species (Şekercioğlu *et al.*, 2025). We assigned all other variables using the dataset curated by (González-Lagos *et al.*, 2021). Briefly, this dataset defines species-level urban and human-disturbed occurrence using IUCN habitat classification data to determine if a species' native range overlaps with modified environments. The urban tolerance index (UTI) is a continuous value using the ratio between individuals of a species occupying an urban area and those occupying surrounding natural environments to account for the dependence of urban occurrence on natural populations bordering urban sites (Evans *et al.*, 2011); (Sol *et al.*, 2017). Large, positive UTI values are thus indicative of species very tolerant

of urban habitat, whereas large, negative UTI values suggest low urban tolerance (Evans *et al.*, 2011); (González-Lagos *et al.*, 2021). Lastly, the exploiter/avoider metric uses community simulations to designate species as urban exploiters or avoiders based on whether they are significantly more or less likely to be abundant in urban environments than expected by chance dispersal from surrounding habitats (Sol *et al.*, 2014). Because the UTI and exploiter/avoider metrics rely on population surveys of bird assemblages across urban gradients, these metrics characterize fewer species in our data (n=743 for habitat breadth and urban or disturbed habitat occurrence, n=274 for urban tolerance, and n=193 for exploiter/avoider status) but represent their tolerance of urban habitats more effectively than occurrence data alone (Sol *et al.*, 2014). We also assigned migratory strategy to each host species per the AVONET database (Tobias *et al.*, 2022). Because of low numbers of host–parasite links associated with partially migratory species (n=205), we collapsed migratory strategy into just two categories: links associated with fully migratory species (n=550), wherein most populations are long-distance migrants, and links associated with non-fully migratory species (n=1,016).

Statistical analysis

To first test for overall congruence between the host and parasite phylogenies and the relative contribution of host–parasite links associated with urban-tolerant hosts to this global fit, we used PACo as implemented with the *paco* package in R (Hutchinson *et al.*, 2017). We constructed a host–parasite association matrix and separate phylogenetic distance matrices for hosts and parasites using the *ape* package (Paradis *et al.*, 2004). Phylogenetic distance matrices were then converted to principal coordinates prior to Procrustes imposition. The sum of squared residuals yielded by the superimposition between the host and parasite principal coordinates (i.e., m_{xy}^2)

indicates their overall congruence, and we used a goodness-of-fit test against 1,000 random permutations of the association matrices to determine if this level of congruence was greater than that expected by chance. To assess the contribution of individual interactions to overall congruence, we used a jackknife procedure to calculate the corrected Procrustes residuals for each host–parasite link. A lower residual value indicates a larger contribution of a given interaction to global cophylogenetic congruence.

We then analyzed these PACo residuals in a series of PGLMMs using the *brms* package (Bürkner, 2017). We averaged residuals per host species and used their log-transformed values as a Gaussian response in three separate sets of 2–6 candidate models. These models differed in the predictor variable used to describe urban tolerance (i.e., urban occurrence, disturbed habitat occurrence, UTI, or exploiter/avoider metric) and additive or interaction effects between migratory strategy and urban tolerance metric (Table 1). Variation inflation factor tests confirmed that migratory strategy was not collinear with any measures of urban tolerance. All models included a host phylogenetic random effect (i.e., via a covariance matrix from the host phylogeny) as well as a fixed effect of number of citations per host species as a proxy for research effort, programmatically collected with the *easyPubMed* package. We ran all PGLMMs using four chains for 20,000 iterations, a burn-in of 50%, and default priors. We verified model convergence by inspecting trace plots and R-hat values. We compared models within each candidate set (Table 1) using the leave-one-out cross-validation information criterion (LOOIC) via the *brms* package, wherein the model with the lowest LOOIC has the highest predictive performance (Vehtari *et al.*, 2015). We then extracted posterior means and 95% credible intervals of model coefficients for all competitive PGLMMs (i.e., within two ΔLOOIC). We repeated each analysis with host residuals restricted to links associated with individual parasite

genera (*Plasmodium*, *Haemoproteus*, or *Leucocytozoon*), because each haemosporidian genus is associated with distinct arthropod vector groups that are expected to respond differently to environmental disturbance (van Hoesel *et al.*, 2019). Since not every host included from the MalAvi database was associated with parasite lineages from all three genera, we only considered urban tolerance variables fully encapsulating the host species in our study (i.e., habitat breadth, urban habitat occurrence, and disturbed habitat occurrence) for these single-genus models. We also repeated analyses with non-collapsed migration strategy categories from AVONET (i.e., fully migratory, partially migratory, and sedentary) to confirm that our methods were not obscuring differences between partially migratory and sedentary species.

RESULTS

The global test of cophylogenetic signal from PACo rejected the null hypothesis of random associations between bird species and their haemosporidian lineages (m_{xy}^2 = 1,049, $p < 0.001$), indicating that haemosporidian evolution overall tracks that of their avian hosts (Figure 3.1). However, we also observed substantial heterogeneity in PACo residuals, suggesting cospeciation is not the sole driver of recorded host–parasite associations (Figure 3.2).

Using our host species–level mean PACo residuals as the response variable in PGLMMs, we found little support for effects of any measure of urban tolerance. Across our three sets of model comparisons (Table 1; habitat breadth and modified habitat occurrence, UTI, and exploiter/avoider status), additive models were always more competitive than models including an interaction effect, indicating that the interaction between urban tolerance and migration was uninformative (Arnold, 2010). Across our most parsimonious models for occurrence, UTI, and exploiter/avoider status, host migration strategy emerged as the only consistently strong predictor

of cophylogenetic congruence. A post-hoc analysis likewise confirmed that these models did not perform any better than simpler models that only included migratory strategy and research effort (Table S3.1). Across our PGLMMs, the credible interval for migration strategy did not overlap zero, with fully migratory species having larger PACo residuals than partially migratory or sedentary species (Figure 3.3, Table 3.2). This suggests that host–parasite links associated with birds that undertake long-distance migrations contribute less to global cophylogenetic congruence.

This effect of long-distance migration on PACo residuals was consistent when analyses were replicated with partially migratory species separated from sedentary species (Tables S3.2 and S3.3). However, this result was not robust to analyses being restricted to a single haemosporidian genus (*Plasmodium*, *Haemoproteus*, or *Leucocytozoon*; Tables S3.4 and S3.5, Figure S1).

DISCUSSION

Ecological traits are well-recognized to mediate co-evolutionary relationships between host species and their parasites (Hafner *et al.*, 1994; Page, 2003; Sweet *et al.*, 2018), but the potential relationship between urban tolerance and cophylogenetic congruence has not been explored. When leveraging a global database of over 2,000 associations between avian hosts and their haemosporidian parasites, we did not find evidence of a relationship between cophylogenetic congruence and various measures of urban tolerance (i.e., habitat breadth, disturbed habitat occurrence, urban habitat occurrence, UTI, and urban exploiter/avoider status). However, we did observe a consistently strong influence of migration strategy on host species–level residuals,

where host–parasite links associated with long-distance migrants contributed less to cophylogenetic congruence between birds and haemosporidia (Figure 3.3).

Migration is hypothesized to weaken cospeciation by exposing migrants to diverse host and parasite communities throughout their annual cycle ("migratory exposure"), increasing host-switching opportunities (Altizer *et al.*, 2011; Koprivnikar and Leung, 2015). Foundational literature on migratory exposure targeted birds and haemosporidian parasites, and subsequent work supports that migratory birds are indeed more frequently infected and tend to host more diverse haemosporidian communities (Figuerola and Green, 2000; Jenkins *et al.*, 2012; de Angeli Dutra *et al.*, 2021b). Yet there is mixed support for migration acting at a macro-evolutionary level to influence cophylogeny between birds and their haemosporidian parasites. Including migratory hosts weakened cophylogenetic congruence between birds and parasites in a study of genus *Leucocytozoon*, but a later study of the genera *Plasmodium* and *Haemoproteus* found that host migration strategy was unimportant to congruence (Jenkins *et al.*, 2012; de Angeli Dutra *et al.*, 2022). Our study expands on the taxonomic breadth of these co-evolutionary studies to consider all three haemosporidian genera simultaneously, ultimately suggesting migration has a weak but non-trivial role in decreasing codivergence between birds and haemosporidian parasites (Figure 3.2, Table 3.2). The effect of migration on host species–level residuals was absent in partially migratory species, consistent at ecological timescales with the finding that partial migrants have more similar haemosporidian prevalence and richness to sedentary species than to fully migratory species (de Angeli Dutra *et al.*, 2021b). Migration also did not emerge as important to congruence when analyses were restricted to any single haemosporidian genus. Migration may therefore have a subtle effect that is only apparent when considering many host–parasite links that are both associated with long-distance migrants and distributed across the

entire haemosporidian phylogeny, overpowering patterns of specialization mediated by ecological differences between *Plasmodium*, *Haemoproteus* and *Leucocytozoon*.

Our results did not support our hypothesis that migration could interact with various measures of anthropogenic tolerance to degrade co-evolutionary links by magnifying the heterogeneity of habitats as well as host and parasite communities encountered throughout the annual cycle. Host switching is strongly influenced by the phylogenetic similarity of prospective hosts to a parasite's current host range, but functional traits that can guide geographic overlap between host and parasite communities, such as migration and urban tolerance, are also essential to consider (Cooper *et al.*, 2012). Host species–level urban tolerance does not seem to contribute to the evolutionary dependence of haemosporidian parasites on their avian hosts. The relatively shallow timescale of urbanization and the lack of relationship host habitat breadth and cophylogenetic congruence suggest this result reflects low co-evolutionary input from the complex of host traits relevant to urban tolerance. However, anthropogenically degraded habitats are nonetheless altering the ecological contexts in which birds and parasites interact. An expanding frontier of research on host–parasite interactions asks how environmental differences could produce rifts between fundamental host specificity, or the theoretical full range of hosts a parasite could use with unrestricted access to any host across space and time, and realized host specificity, the measurable range of hosts a parasite is associated with in a particular spatiotemporal context (Wells and Clark, 2019). For example, the host specificity of identical haemosporidian cytochrome *b* lineages in the genera *Plasmodium* and *Haemoproteus* can vary across avian communities in response to regional rainfall patterns (Fecchio *et al.*, 2019). Anthropogenic disturbance is an increasingly common landscape feature that can shift the composition of hosts available to parasites and may also weaken or strengthen physiological

barriers (e.g., host immune defense) to colonizing new hosts. Analytical methods capable of exploring host–parasite interactions at finer ecological scales, such as species interaction networks, may be useful for generating new insights on how habitat degradation could shape associations between birds and their haemosporidian parasites (Poisot *et al.*, 2015; Blasco-Costa *et al.*, 2021). Insights generated about the relationship between parasite specialization and urbanization at ecological scales will become increasingly relevant at co-evolutionary scales as host and parasite taxa respond to accelerating anthropogenic change.

This study is the first to our knowledge to assess the contribution of avian host traits to cophylogenetic congruence spanning the entire known diversity of avian haemosporidian parasites (genera *Plasmodium*, *Haemoproteus*, and *Leucocytozoon*). In addition to differences in pathogenicity, the three haemosporidian genera undergo the sexual phase of their life cycle in different arthropod vector families (Culicidae, Ceratopogonidae, Simuulidae and rarely Hippoboscidae; Valkiunas, 2004)). Haemosporidian genus–specific differences in vector ecology should be considered alongside host traits, because such differences are suspected to be strong filters to avian–haemosporidian interactions, including realized host specificity (Fecchio *et al.*, 2019). Birds tolerant of anthropogenically disturbed habitats should be exposed to arthropod vector communities that also tolerate or favor disturbed habitats, based on factors such as urban heat island effects and the accumulation of stagnant water (Araujo *et al.*, 2015). For example, mosquito abundance, species richness, and community composition can differ across rural–urban gradients, with evidence that natural and disturbed habitats can harbor the greatest mosquito abundance and richness while urbanized habitats attract high densities of urban-adapted species (Ferraguti *et al.*, 2016). However, this pattern is not expected to be consistent across every haemosporidian vector. Both mosquitoes and biting midges, vectors of haemosporidia in the

genera *Plasmodium* and *Haemoproteus*, have microhabitat requirements that can be accommodated by disturbed habitats (van Hoesel *et al.*, 2019). Conversely, black fly vectors of haemosporidia in the genus *Leucocytozoon* are reliant on clean, running water and sufficient vegetation cover, and are adapted for development and transmission in relatively cool environments (Suri *et al.*, 2017; Fecchio *et al.*, 2020). Increasing our understanding of how urbanization and species-level urban tolerance could affect host switching events will require community-level studies to interrogate how habitat disturbance affects host specificity from the multi-faceted perspective of avian hosts, haemosporidian lineages, and arthropod vectors.

We note several limitations to our approach. First, haemosporidian lineages in the genus *Leucocytozoon* are less represented in the MalAvi database (host associations $n = 205$) than lineages in the genera *Plasmodium* and *Haemoproteus* (host associations $n = 454$ and $n = 398$, respectively). This bias may stem from the high pathogenicity of some lineages of *Plasmodium* (e.g., *Plasmodium relictum*; Dadam *et al.*, 2019) as well as the additional diagnostic steps required to detect *Leucocytozoon* infections with commonly used primer sets (Bensch *et al.*, 2009). Second, although we used a filtering process to avoid including tenuous host associations, cytochrome *b* sequences associated with a given avian host in MalAvi are not necessarily linked to the presence of circulating gametocytes that can verify infection transmissibility. In other words, the high sensitivity of PCR diagnostics that are not coupled with microscopy means documented host–parasite associations could be based on parasite genetic material from dead-end infections rather than competent hosts (Pigeault *et al.*, 2015; Martin *et al.*, 2016). The effects of anthropogenic tolerance on host–parasite associations may be better understood when also accounting for host competence (Gervasi *et al.*, 2015). In addition, urban tolerance is a dynamic trait that can shift throughout a migratory bird's annual cycle (Callaghan *et al.*, 2021). The

potential role of urban tolerance in haemosporidian parasite diversification may be better represented by strictly accounting for urban tolerance during the part of the annual cycle birds overlap with haemosporidian parasites and vectors (e.g. predominantly the breeding season in temperate migrants; Valkiunas, 2004).

Urbanization is rapidly and drastically transforming the planet and the landscapes hosting its biodiversity, with the potential to alter host–parasite associations. We here tested the hypothesis that a bird species' tolerance of anthropogenically modified habitats corresponds with decreased host specialization strong enough to degrade associations between birds and their haemosporidian parasites over evolutionary time. Host species tolerance of heterogeneous or disturbed habitats did not impact how strongly their haemosporidian parasites tracked them over evolutionary time. However, haemosporidian diversification with migratory species was less strongly characterized by cospeciation, indicating that migration strategy may facilitate host switching. Despite finding minimal effects of urban tolerance on host switching over evolutionary time, we note that 73% of migratory species represented in our study overlap with disturbed and/or urbanized habitats throughout some period of their annual cycle. As an urbanizing world will promote greater interactions among migratory and non-migratory bird species and their vectors (Satterfield *et al.*, 2018; Partridge and Clark, 2018; Ramírez-Cruz *et al.*, 2020), our results encourage further study of how migration shapes haemosporidian evolution as well as community-level interrogations of how habitat disturbance impacts host switching.

TABLES

Table 3.1. Comparisons of PGLMMs differing in the metric of urban tolerance used (urban or disturbed habitat occurrence, urban tolerance index, or urban exploiter/avoider status). Model fit was assessed using LOOIC and corresponding standard error (SE), with the best-performing models represented by the lowest LOOIC. All models additionally include a phylogenetic random effect computed using the host phylogeny covariance matrix.

Model comparison	**Fixed effects**	**Model weight**	**ΔLOOIC ± *SE***
Urban habitat occurrence, disturbed habitat occurrence, and habitat breadth (*n=743*)	Disturbed occurrence + Migration strategy + Research effort	0.31	0 ± 54.71
	Habitat breadth + Migration strategy + Research effort	0.20	0.89 ± 55.03
	Urban occurrence + Migration strategy + Research effort	0.20	0.90 ± 54.84
	Urban occurrence * Migration strategy + Research effort	0.11	2.12 ± 54.68
	Habitat breadth * Migration strategy + Research effort	0.09	2.40 ± 55.28
	Disturbed occurrence * Migration strategy + Research effort	0.09	2.46 ± 54.67
Urban tolerance index (*n=274*)	Urban tolerance index + Migration strategy + Research effort	0.74	0 ± 26.24
	Urban tolerance index * Migration strategy + Research effort	0.26	2.04 ± 26.33
Urban exploiter/avoider status (*n=193*)	Urban exploiter/avoider status + Migration strategy + Research effort	0.88	0 ± 23.36
	Urban exploiter/avoider status * Migration strategy + Research effort	0.12	4.03 ± 23.25

TABLES

Table 3.2. Posterior means and 95% credible intervals for fixed effects in the top PGLMM for each of our three model comparisons (disturbed and urban habitat occurrence, urban tolerance index, and urban exploiter/avoider status). Bold indicates model coefficients where credible intervals do not overlap zero.

Model	**Coefficient**	**Posterior mean**	**95% credible interval**
Disturbed habitat occurrence + Migration strategy + Research effort	Disturbed habitat occurrence	-0.002	-0.01─0
	Migration strategy	0.014	0.006–0.022
	Number of citations	0	0─0
Urban tolerance index + Migration strategy + Research effort	Urban tolerance index	-0.005	-0.03–0.02
	Migration strategy	0.004	0.004-0.027
	Number of citations	-0.001	-0.004–0.002
Urban exploiter/avoider status + Migration strategy + Research effort	Urban exploiter/avoider status	0.007	-0.006–0.020
	Migration strategy	0.023	0.008–0.038
	Number of citations	-0.001	-0.005─0.003

FIGURES

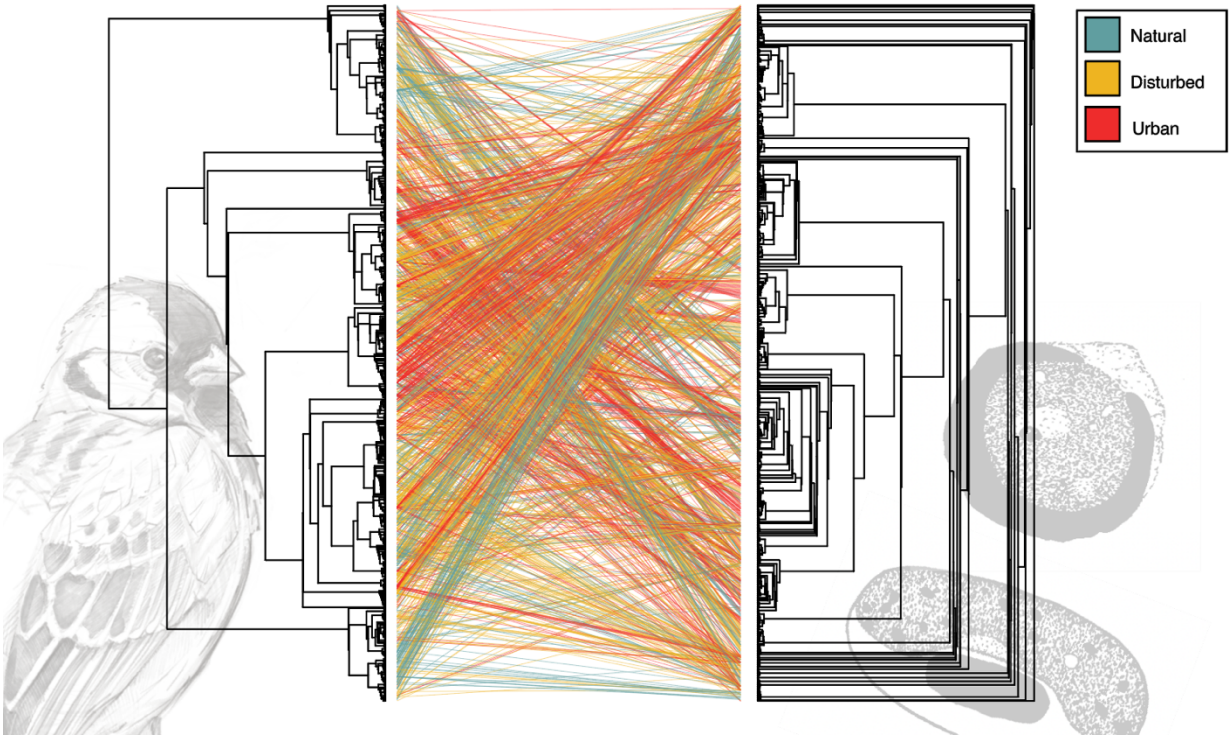


Figure 3.1. Tanglegram depicting host–parasite links between a phylogeny of birds (left) and of their haemosporidian parasites in the genera *Plasmodium*, *Haemoproteus* and *Leucocytozoon* (right). Associations are colored to correspond with host species occurrence in modified habitats, with yellow links representing species occurring in anthropogenically disturbed habitats, red links representing species occurring in the most "severe" category of disturbance (urbanization), and green links representing species that are excluded from disturbed habitats. Illustrations are by Javier Lazaro (left) and Gediminas Valkiūnas (right).

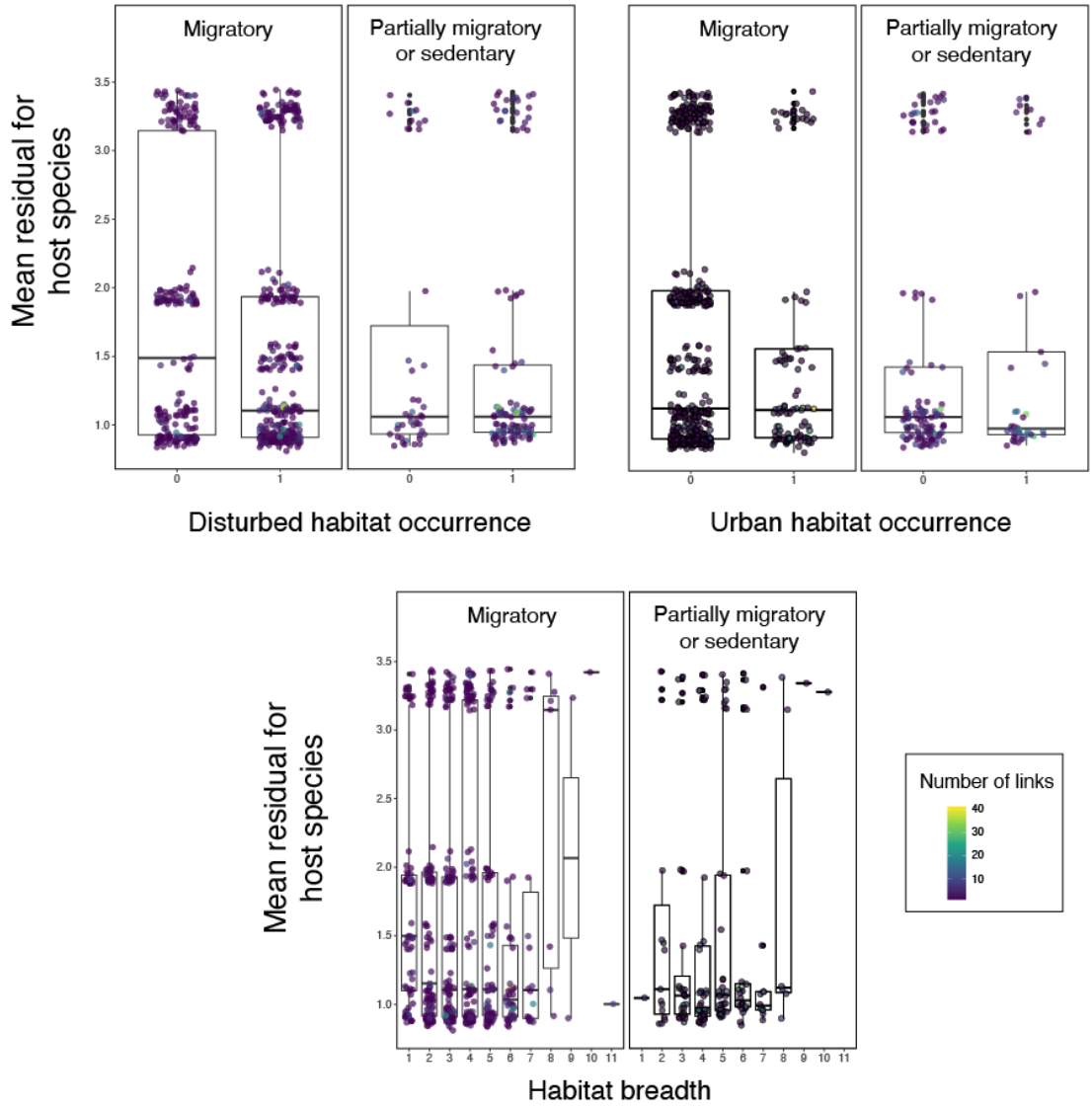


Figure 3.2. Mean jackknifed host species–level PACo residuals derived from bird–haemosporidian links, stratified by a species' migration strategy (fully migratory or non-fully migratory) and the urban tolerance variables representing every avian host in our study (i.e., disturbed habitat occurrence, urban habitat occurrence, and habitat breadth). Points are shaded by the number of links representing a given bird-haemosporidian association in the MalAvi database and jittered to reduce overlap.

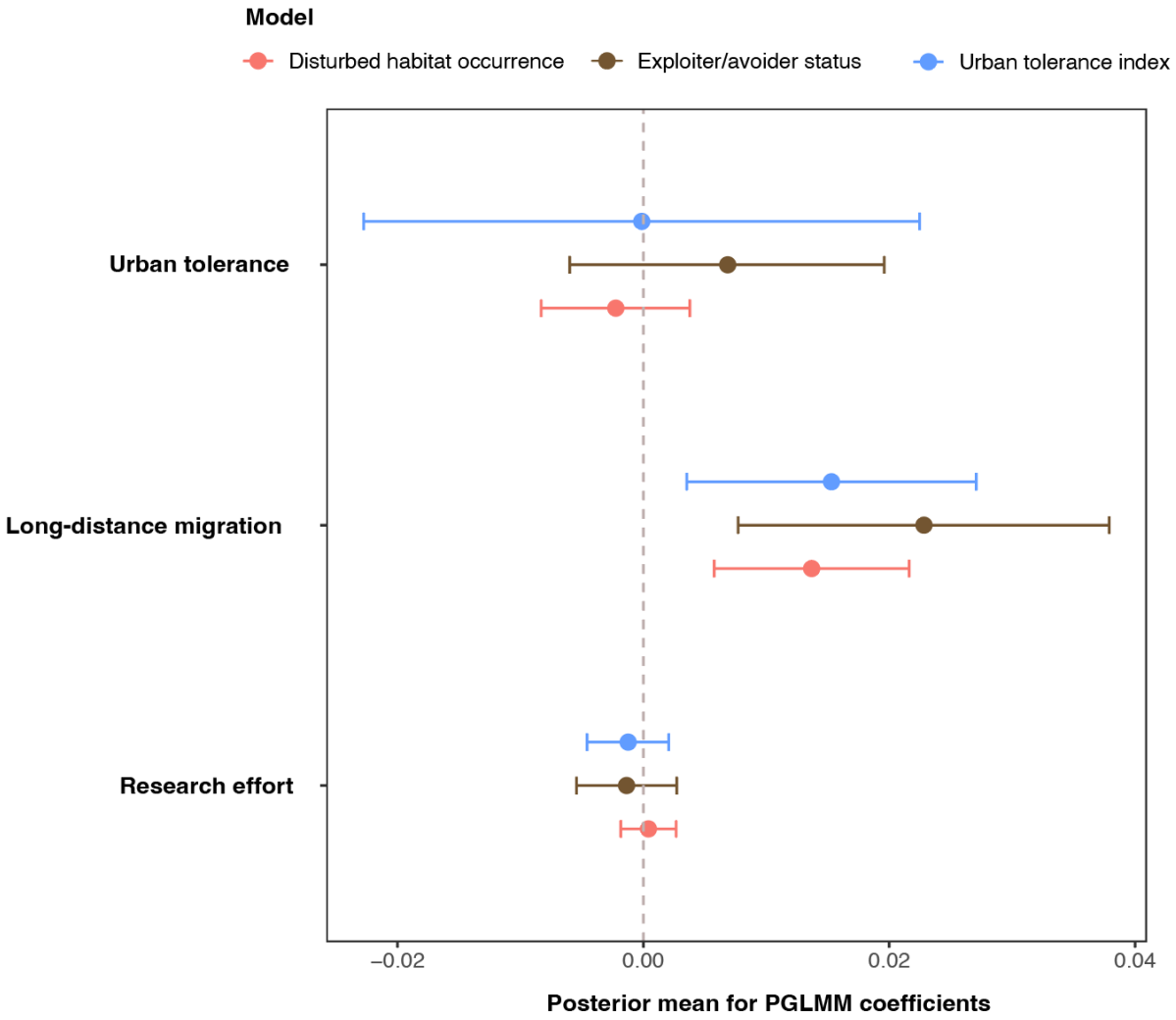


Figure 3.3. Posterior means and 95% credible intervals for fixed effects in the most competitive PGLMMs of log-transformed mean jackknifed PACo residuals, which inform the contribution of host–parasite links to global cophylogenetic congruence (larger residual = less contribution to co-speciation). Predictors with credible intervals that do not overlap zero are assumed to have a strong effect.

SUPPLEMENTARY TABLES AND FIGURES

Table S3.1. Comparisons of PGLMMs differing in the metric of urban tolerance used (urban or disturbed habitat occurrence, urban tolerance index, or urban exploiter/avoider status), compared against a minimal model including only migration strategy and research effort. Model fit was assessed using LOOIC and corresponding standard error (SE), with the best-performing models represented by the lowest LOOIC. All models additionally include a phylogenetic random effect computed using the host phylogeny covariance matrix.

Model comparison	**Fixed effects**	**Model weight**	**ΔLOOIC ± *SE***
Urban habitat occurrence, disturbed habitat occurrence, and habitat breadth (*n=743*)	Migration strategy + research effort	**0.34**	**0 ± *54.89***
	Disturbed occurrence + Migration strategy + Research effort	0.21	0.98 ± *54.71*
	Habitat breadth + Migration strategy + Research effort	0.13	1.87 ± *55.03*
	Urban occurrence + Migration strategy + Research effort	0.13	1.88 ± *54.84*
	Urban occurrence * Migration strategy + Research effort	0.07	3.10 ± *54.68*
	Habitat breadth * Migration strategy + Research effort	0.06	3.38 ± *55.28*
	Disturbed occurrence * Migration strategy + Research effort	0.06	3.44 ± *54.67*

SUPPLEMENTARY TABLES AND FIGURES

Table S3.1 (*continued*).

Model comparison	**Fixed effects**	**Model weight**	**ΔLOOIC ± *SE***
Urban tolerance index (*n=274*)	Migration strategy + Research effort	**0.70**	**0 ± 26.35**
	Urban tolerance index + Migratory strategy + Research effort	0.18	2.72 (26.26)
	Urban tolerance index * Migration strategy + Research effort	0.12	3.62 (26.41)
Urban exploiter/avoider status (*n=193*)	Migration strategy + Research effort	**0.47**	0 ± 23.33
	Urban exploiter/avoider status + Migration strategy + Research effort	0.42	0.24 ± *23.37*
	Urban exploiter/avoider status * Migration strategy + Research effort	0.11	2.85 ± *23.35*

SUPPLEMENTARY TABLES AND FIGURES

Table S3.2. Comparisons of PGLMMs differing in the metric of urban tolerance used (urban or disturbed habitat occurrence, urban tolerance index, or urban exploiter/avoider status), with migration strategy preserved as AVONET categories (fully migratory, partially migratory, or sedentary). Model fit was assessed using LOOIC and corresponding standard error (SE), with the best-performing models represented by the lowest LOOIC. All models additionally include a phylogenetic random effect computed using the host phylogeny covariance matrix.

Model comparison	**Fixed effects**	**Model weight**	**ΔLOOIC ± *SE***
Urban habitat occurrence, disturbed habitat occurrence, and habitat breadth (*n*=743)	Habitat breadth + AVONET migration strategy + Research effort	**0.61**	**0 ± 54.76**
	Habitat breadth * AVONET migration strategy + Research effort	**0.35**	**1.10 ± 55.05**
	Urban occurrence + AVONET migration strategy + Research effort	0.04	5.42 ± 54.68
	Disturbed occurrence + Migratory strategy + Research effort	0.35	5.70 ± 54.49
	Disturbed occurrence * Migratory strategy + Research effort	0.04	6.79 ± 54.86
	Urban occurrence * AVONET migration strategy + Research effort	0.61	10.59 ± 55.15
Urban tolerance index (*n*=274)	Urban tolerance index + AVONET migration strategy + Research effort	0.83	0 ± 26.47
	Urban tolerance index * AVONET migration strategy + Research effort	0.17	3.21 ± 26.22
Exploiter/avoider status (*n*=193)	Exploiter/avoider status + AVONET migration strategy + Research effort	0.79	0 ± 22.96
	Exploiter/avoider status * AVONET migration strategy + Research effort	0.21	2.62 ± 22.26

SUPPLEMENTARY TABLES AND FIGURES

Table S3.3. Posterior means and 95% credible intervals for fixed effects in the top PGLMM for each of our three model comparisons (disturbed and urban habitat occurrence, urban tolerance index, and urban exploiter/avoider status), with migration strategy preserved as AVONET categories (fully migratory, partially migratory, or sedentary).

Model	**Coefficient**	**Posterior mean**	**95% credible interval**
Habitat breadth + AVONET migration strategy + Research effort	Habitat breadth	-0.0002	-0.001─0.002
	Partial migration	-0.0039	-0.005─0.013
	Long–distance migration	-0.0148	0.006─0.023
	Number of citations	0	0.002─0.002
Urban tolerance index + AVONET migration strategy + Research effort	Urban tolerance index	0	-0.023─0.023
	Partial migration	0.0014	-0.013─0.016
	Long–distance migration	0.1579	0.003─0.029
	Number of citations	-0.0013	-0.005─0.002
Exploiter/avoider status + AVONET migration strategy + Research effort	Exploiter/avoider status	0.0065	-0.006─0.020
	Partial migration	0.0082	-0.013─0.019
	Long–distance migration	0.0241	0.007─0.041
	Number of citations	-0.0014	-0.005─0.003

SUPPLEMENTARY TABLES AND FIGURES

Table S3.4. Comparisons of PGLMMs differing in the metric of urban tolerance used (urban or disturbed habitat occurrence, urban tolerance index, or urban exploiter/avoider status) and the haemosporidian genus represented by the host–parasite residuals used to calculate the response. Model fit was assessed using LOOIC and corresponding standard error (SE), with the best-performing models represented by the lowest LOOIC. All models additionally include a phylogenetic random effect computed using the host phylogeny covariance matrix.

Haemosporidian genus	**Fixed effects**	**Model weight**	**ΔLOOIC ± *SE***
Leucocytozoon (*n*=205 host species)	Habitat breadth + Migratory strategy + Research effort	**0.42**	0 ± *53.77*
	Urban occurrence * Migratory strategy + Research effort	0.36	0.30 ± 51.08
	Urban occurrence + Migratory strategy + Research effort	0.16	1.77 ± 52.14
	Disturbed occurrence + Migratory strategy + Research effort	0.04	4.79 ± 54.82
	Habitat breadth * Migratory strategy + Research effort	0.01	6.79 ± 55.08
	Disturbed occurrence * Migratory strategy + Research effort	<0.01	8.67 ± 56.26
Haemoproteus (*n*=398 host species)	Urban occurrence * Migratory strategy + Research effort	0.58	0 ± 63.63
	Urban occurrence + Migratory strategy + Research effort	0.29	1.40 ± 63.95

Table S3.4 (*continued*).

Haemoproteus (*n*=398 host species)	Disturbed occurrence + Migratory strategy + Research effort	0.07	4.28 ± 65.77
	Habitat breadth + Migratory strategy + Research effort	0.04	5.64 ± 65.73
	Habitat breadth * Migratory strategy + Research effort	0.02	6.59 ± 65.14
	Disturbed occurrence * Migratory strategy + Research effort	<0.01	10.32 ± 67.70
Plasmodium (n = 454 host species)	Habitat breadth * Migratory strategy + Research effort	0.33	0 ± 38.70
	Habitat breadth + Migratory strategy + Research effort	0.31	0.12 ± 39.47
	Disturbed occurrence + Migratory strategy + Research effort	0.15	1.58 ± 39.01
	Disturbed occurrence * Migratory strategy + Research effort	0.12	2.03 ± 38.83
	Urban occurrence + Migratory strategy + Research effort	0.05	3.69 ± 39.93
	Urban occurrence * Migratory strategy + Research effort	0.03	4.56 ± 39.22

SUPPLEMENTARY TABLES AND FIGURES

Table S3.5. Posterior means and 95% credible intervals for fixed effects in the top PGLMM for each of our three model comparisons (disturbed and urban habitat occurrence, urban tolerance index, and urban exploiter/avoider status), divided by haemosporidian genus (*Plasmodium*, *Haemoproteus* or *Leucocytozoon*).

Haemosporidian genus	**Model**	**Coefficient**	**Posterior mean**	**95% credible interval**
Leucocytozoon	Habitat breadth + Migratory strategy + Research effort	Habitat breadth	0	-0.002–0.002
		Migratory strategy	0	-0.007–0.006
		Research effort	0	-0.003–0.0003
Haemoproteus	Urban occurrence * Migratory strategy + Research effort	Urban occurrence * Migratory strategy	0.0037	-0.0011–0.003
		Research effort	-0.0003	-0.001–0.001
Plasmodium	Habitat breadth + Migratory strategy + Research effort	Habitat breadth	0.0007	-0.0008–0.0011
		Migratory strategy	-0.0003	-0.0068–0.0024
		Research effort	-0.00005	-0.0013–0.0012

SUPPLEMENTARY TABLES AND FIGURES

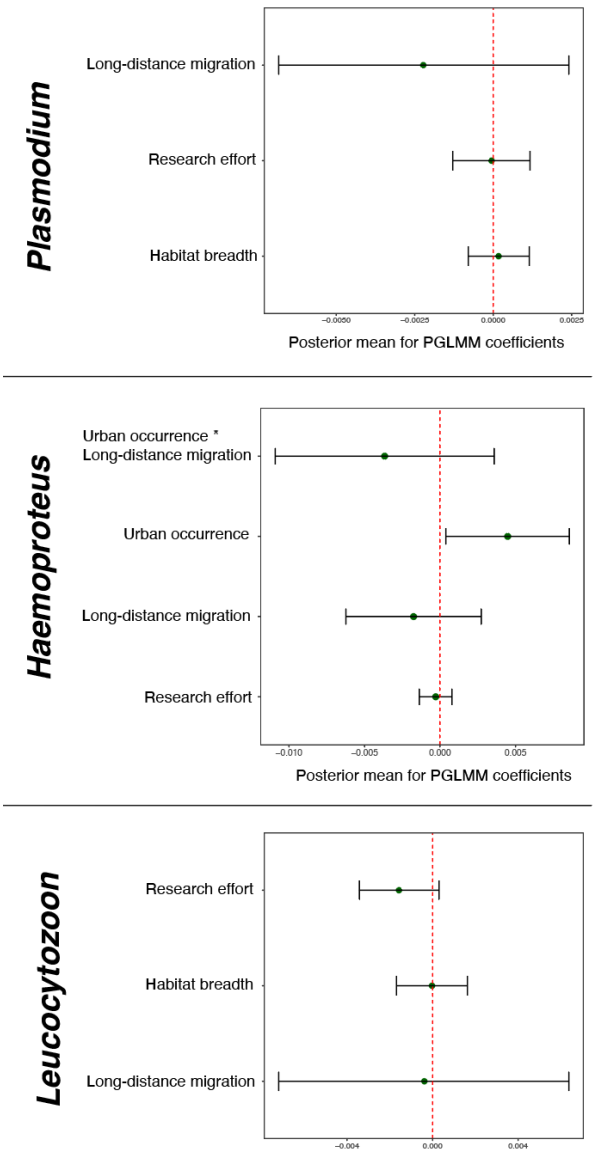


Figure S3.1. Posterior means and 95% credible intervals for fixed effects in the most competitive PGLMMs of log-transformed mean jackknifed PACo residuals for links associated with each haemosporidian genus (*Plasmodium*, *Haemoproteus*, or *Leucocytozoon*), which inform the contribution of host–parasite links to global cophylogenetic congruence (larger residual = less contribution to co-speciation). Predictors with credible intervals that do not overlap zero are assumed to have a strong effect.

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