

Research Article

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Author for correspondence:

Dany Garant, E-mail: Dany.Garant@USherbrooke.ca

Environmental determinants of haemosporidian parasite prevalence in a declining population of Tree swallows

Audrey Turcotte, Marc Bélisle, Fanie Pelletier and Dany Garant

Département de biologie, Université de Sherbrooke, 2500 boulevard de l'Université, Sherbrooke, QC, J1K 2R1, Canada

Abstract

The prevalence of vector-borne parasites such as haemosporidian species is influenced by several environmental factors. While the negative effects of parasitism on hosts are well documented, these can also be amplified by interactions with environmental stressors, many of which are anthropogenic. Yet, we know little about the possible effects of anthropogenic perturbations on parasite prevalence. The goals of this study were to assess the prevalence and environmental determinants of haemosporidian parasites in a declining population of Tree swallows (*Tachycineta bicolor*) living in an agricultural landscape in southern Québec, Canada. Overall, a low prevalence and a moderate lineage diversity were identified in both adults and nestlings, confirming that transmission can occur during the breeding period. Anthropogenic areas, extensive cultures (hayfields and pastures) and forest cover within 500 km of nest boxes, as well as daily temperature fluctuations, were all related to infection by haemosporidian parasites. These findings suggest that anthropogenic alterations of landscape composition can modulate the prevalence of haemosporidian parasites in Tree swallows. Our results represent a baseline for future comparative studies assessing haemosporidian parasite prevalence in human-modified landscapes.

Introduction

Parasitism is an important factor influencing population dynamics of wild hosts (Moss *et al.* 1982; Price *et al.* 1986). Indeed, parasitism can impact individual fitness through its effects on various components of a host's life such as its reproductive success and survival (Valkiūnas, 2005; Holmstrup *et al.* 2010). Parasite transmission is driven by complex interactions among diverse environmental variables that are generally heterogeneous in the landscape (Lachish *et al.* 2011). Therefore, to better understand and predict the dynamics of parasite infection in wild hosts, it is crucial to assess the effects of environmental factors on prevalence at a fine spatial scale (Gonzalez-Quevedo *et al.* 2014). Furthermore, for host species with large-scale migration patterns, documenting the extent of spatial heterogeneity in parasite prevalence at the population level may also provide some key insights about transmission dynamics (Bensch and Åkesson, 2003; Knowles *et al.* 2014). A homogeneous distribution of parasites prevalence on breeding sites generally suggests a transmission occurring after post-reproduction dispersal on wintering grounds. Alternatively, a more heterogeneous distribution should suggest a transmission occurring on breeding sites, which would reflect the influence of local environment variables on vectors (Knowles *et al.* 2014).

In birds, several environmental factors such as climate, landscape composition and avian population density and diversity have been shown to affect the spatial distribution of avian vector-borne parasites (Sérandour *et al.* 2007; Galardo *et al.* 2009; Okanga *et al.* 2013). In general, the geographic distribution of parasites will usually reflect vector abundance, which can be modulated by environmental conditions throughout the landscape (Hellgren, 2005; Loiseau *et al.* 2010; Grillo *et al.* 2012; Ellis *et al.* 2015). For instance, higher temperatures may increase (especially for *Plasmodium* spp.) the rate of parasite development in vectors, allowing them to reach their infectious stage before the mosquitoes' next blood meals, which should promote parasite transmission (Paaajmans *et al.* 2009; Garamszegi, 2011). Other studies have shown that high vector–host encounter rates are generally associated with a higher host density (Fourcade *et al.* 2014; Ellis *et al.* 2017). Characteristics of landscape composition, such as proximity to water bodies, also can be important factors affecting the development of some vectors because of habitat-specific phases of their lifecycles (Wood *et al.* 2007; Lachish *et al.* 2011; Okanga *et al.* 2013).

Negative effects of parasitism can be amplified by interactions with environmental stressors of anthropogenic origin, such as habitat loss or exposure to contaminants (Clinchy *et al.* 2004; Coors *et al.* 2008; Holmstrup *et al.* 2010; Dunn *et al.* 2013). These potentially negative synergistic interactions can be particularly detrimental for declining wild populations that may already show signs of reduced immunological capacity and/or increased physiological demands (Clinchy *et al.* 2004; Sih *et al.* 2004). For example, agricultural intensification,

which results in landscape homogenization and increased use of pesticides, appears to be responsible for the decline in quality and quantity of breeding habitats for several farmland birds (Jobin *et al.* 1996; Benton *et al.* 2002; Chiron *et al.* 2014). Pesticide applications during the breeding season can indeed play an important role by altering the immune system and reducing the breeding success and survival of farmland birds, particularly for insectivores through both toxicological and trophic effects (Benton *et al.* 2002; Hart *et al.* 2006; Chiron *et al.* 2014; Hallmann *et al.* 2014; Lopez-Antia *et al.* 2015). Given the possible negative interaction with pesticides, parasitism may play a major role in the decline of farmland birds in agricultural landscapes (Dunn *et al.* 2013). Yet, to our knowledge, no studies have assessed the influence of agricultural intensification, as a proxy of pesticide use, on the prevalence of vector-borne parasites in declining populations of insectivorous birds (Dunn *et al.* 2013; see also: Zehntindjiev *et al.* 2012a).

Avian haemosporidians are vector-borne blood parasites that cause a malaria-like disease. This group of parasitic protists is ubiquitous and abundant throughout avian species (Valkiūnas, 2005). Parasites of three genera (*Haemoproteus* spp., *Plasmodium* spp. and *Leucocytozoon* spp.) commonly infect birds and differ in some aspects of their life cycle and vectors (e.g. *Haemoproteus* species main vectors: Hippoboscidae and Ceratopogonidae; *Plasmodium*: Culicidae; *Leucocytozoon*: Simuliidae) (Valkiūnas, 2005; Marzal, 2012). Previous studies have identified negative effects of haemosporidian parasites on body condition, breeding success and survival of many bird species (Dawson and Bortolotti, 2000; Hatchwell *et al.* 2001; Navarro *et al.* 2003; Marzal *et al.* 2008; Knowles *et al.* 2010; Lachish *et al.* 2011). For instance, Asghar *et al.* (2015) showed long-lasting effects of avian malaria (*Plasmodium* and *Haemoproteus* spp.), such as reduced lifespan and lifetime number of offspring, in Great reed warblers (*Acrocephalus arundinaceus*). Moreover, the prevalence of haemosporidian parasites has been shown to depend upon environmental conditions (Okanga *et al.* 2013; Gudex-Cross *et al.* 2015; Hernández-Lara *et al.* 2017). For instance, Okanga *et al.* (2013) identified higher avian malaria prevalence in wetland habitats related to the presence of rich nutrient sources and suitable breeding site for vector development. However, the effects of human-driven environmental changes on haemosporidian parasite prevalence, particularly for *Leucocytozoon* species, have never been addressed within an agricultural context.

This study aims to identify the environmental factors affecting haemosporidian parasite prevalence and patterns of transmission in a declining wild population of Tree swallows (*Tachycineta bicolor*), in southern Québec, Canada. More specifically, our

objectives are to: (1) quantify the prevalence and diversity of haemosporidian parasites in this population using a large sample size collected over 4 years; (2) determine if transmission occurs during the breeding season; and (3) identify environmental variables, such as climate, landscape composition, as well as avian population density and diversity, associated with the infection status of individuals within this population. The Tree swallow is a small passerine bird that migrates annually between its wintering grounds in the southern USA and Central America to its breeding sites over much of North America (Winkler *et al.* 2010). This aerial insectivore nests in secondary cavities in open areas such as pastures and agricultural fields. The studied population is located within a gradient of agricultural intensification that was previously linked to variation in the density of its main prey (i.e. Diptera; Rioux Paquette *et al.* 2013). Tree swallow populations are declining in northeastern North America (Michel *et al.* 2016), and our studied population is no exception (Rioux Paquette *et al.* 2014).

METHODS

Study system and bird sampling

This study is the part of a long-term project that monitors a breeding population of Tree swallows in southern Québec (Canada) since 2004. The study system, characterized by an east-west gradient of agricultural intensification, covers an area of 10 200 km² and consists of 400 nest boxes uniformly distributed among 40 farms [Fig. 1; see Ghilain and Bélisle (2008) for details on the study system]. All nest boxes were visited every 2 days during the breeding season (early May to mid-July). Breeding females and males were captured directly in the nest box during incubation and food provisioning, respectively. For this study, 1150 adult Tree swallows were sampled between 2012 and 2015 (females = 687, males = 463). Nestlings were captured directly in the nest at 16 days old in 2015 on six farms (see Fig. 1). These farms were selected because infected adults were detected at those locations between 2012 and 2014. We determined the sex of adult birds in the field by the presence of a brood patch and subsequently confirmed it with molecular techniques [see Lessard *et al.* (2014) for details]. At first capture, each bird was identified with a unique US Fish and Wildlife service aluminium band. A blood sample was taken from the brachial vein of each individual on a qualitative P8 grade filter paper (Thermo Fisher Scientific). Blood samples were dried and stored at room temperature until DNA extractions. For individuals found dead in the study system, a piece of tissue (25 mm²) at

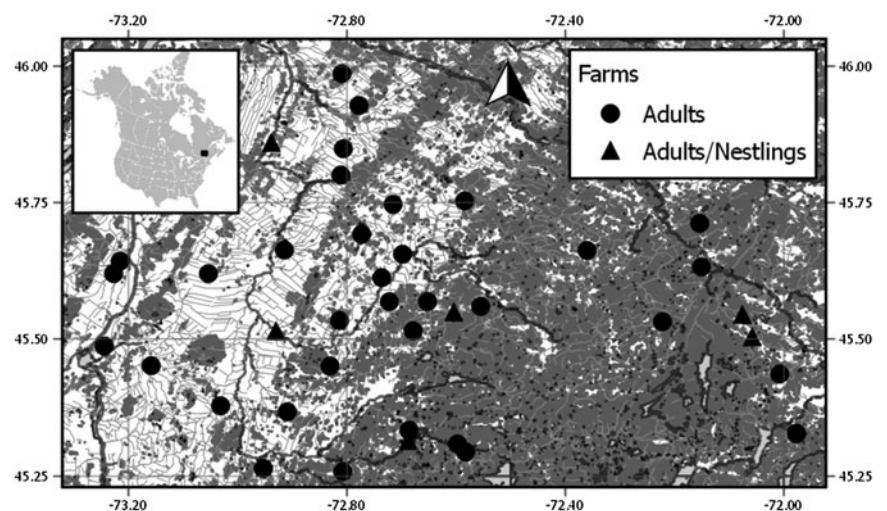


Fig. 1. Map of the 40 farms (black circle and triangle) used to sample Tree swallows in southern Québec, Canada. Land cover types are based on a mosaic of classified Landsat-TM satellite images (Canadian Wildlife Service, 2004) and include forest cover (dark grey), and intensive cultures (white). Water bodies are shown in light grey. Coordinates are in decimal degrees. This map was created with QGIS 2.0 (QGIS Team Development, 2016)

the base of a leg was taken for DNA extractions and preserved in 70% ethanol. All manipulations were approved by the Université de Sherbrooke Animal Care Committee (protocol number DG 2010-01 and DG 2014-01).

Molecular analyses

DNA was extracted from blood samples with an overnight proteinase K digestion followed by a salt extraction (Aljanabi and Martinez, 1997). Extracted DNA samples were then checked and quantified on 1% agarose gels. The detection of haemosporidian parasites from bird blood samples was performed with a nested polymerase chain reaction (PCR), which consists of two successive PCR amplifications (Hellgren *et al.* 2004). The first amplification targets a conserved section of mitochondrial DNA common to parasites of the three genera we studied with the following primers pairs: HaemNF1/HaemNR3 [designed by Hellgren *et al.* (2004)]. This first step increases the performance of the second PCR, which has specific primers for the amplification of *Plasmodium* and *Haemoproteus* species together: HaemF/HaemR2 and *Leucocytozoon* species separately: HaemFL/HaemR2L (Hellgren *et al.* 2004). The first PCR was performed in a total volume of 25 μL containing 40–60 ng of genomic DNA, 10 mM Tris-HCl (pH = 9), 50 mM KCl, 0.1% Triton X-100, 2.5 mM MgCl_2 , 0.1 mM dNTPs, 0.4 mM of each primer and 1 U μL^{-1} AmpliTaq Gold (Applied Biosystems). The second PCR was performed with similar conditions, except that we used 5 μL of the first PCR product instead of genomic DNA. The thermal profile for both PCRs consisted of an initial denaturation of 2 min at 94 °C followed by 35 cycles of 30 s at 94 °C, 30 s at 50 °C and 45 s at 72 °C and a final elongation period of 10 min at 72 °C. To determine if the PCR amplification was successful, 5 μL of the second PCR product was migrated on a 2% agarose gel stained with ethidium bromide and visualized under UV light. Each batch of PCR amplification contained a positive control (i.e. DNA of a known infected individual) and a negative control (i.e. H_2O) to control for possible contamination or problems during manipulations (Szöllösi *et al.* 2008). The presence of an infection was confirmed by the detection of an amplification at ca. 500-bp (478-bp for *Leucocytozoon* species and 480-bp for *Plasmodium* and *Haemoproteus* species without primers).

Sequencing and identification of lineages

PCR products from all positive amplifications were sent to the Plateforme de séquençage et de génotypage du Centre de Recherche du CHUL (CHUQ, Québec, Canada) for sequencing with an AB 3730xl Data Analyser (Applied Biosystems). Obtained sequences were compared with those present in the MalAvi database of haemosporidian avian parasites, which contains more than 2400 lineages (Bensch *et al.* 2009). Identified sequences were aligned with their closest sequence matches in MalAvi and analysed using MEGA version 7 (Kumar *et al.* 2016). The electropherograms of each sequence were visually checked for the presence of sites with double base calling suggesting a mixed infection. All samples with potential mixed infections were re-amplified and re-sequenced to validate the presence of mixed infections and identify the lineage of the additional infection [see Matthews *et al.* (2015) for details on this approach].

Landscape characterization

We characterized the proportion of different land covers around each nest box at a large (5-km radius) and a fine (500-m radius) spatial scale. These 5-km and 500-m radii encompass,

respectively, the area used by Tree swallows during the food-provisioning period (Ghilain and Bélisle, 2008) and the local environment of the farm (Lagrange *et al.* 2014). Land cover at the 5-km scale was estimated using a mosaic of geo-referenced and classified Landsat-7 satellite images taken between August 1999 and May 2003 (Canadian Wildlife Service, 2004) and ArcView GIS spatial analyst 2.0a (ESRI, 2005). At the 500-m scale, land cover was assessed annually in the field by determining visually the crop growing in each field polygon delineated with orthophotos (scale: 1:40 000). Land cover was classified in five categories at both scales: (1) intensive cultures (e.g. corn and soya-bean), (2) extensive cultures (e.g. pastures and perennial cultures, such as various grasses, alfalfa and clover), (3) water bodies, (4) forested areas and (5) anthropic areas (e.g. buildings and roads). We also conducted livestock (i.e. number of horses, cattle and sheep) counts at the beginning of each farm visit within a 500-m radius from the central point of the farm. These counts were then averaged across visits for each farm and livestock category.

Weather variables

Temperature and precipitation data were collected on each farm every year. Each weather variable was obtained relative to each bird sampled by using data collected between the start of the field season and the date of the blood sampling. Thus, each weather variable reflected the conditions that may have affected the infection status of each bird prior to blood sampling. Temperature (± 0.5 °C) was taken every hour with a temperature data logger (Thermocron iButton DS 1992 L; Dallas Semiconductor, Dallas, Texas, USA) installed under the central nest box on each farm. We used five different temperature variables: (1) mean daily temperature, (2) mean diurnal temperature (6 AM to 8 PM, inclusively), (3) mean minimum daily temperature, (4) mean maximum daily temperature and (5) mean daily temperature variation (i.e. mean maximum daily temperature – mean minimum daily temperature). Precipitation data (± 0.5 mm) were obtained on a 48-h basis using pluviometers installed on each farm.

Bird population density and diversity index

We used multiple indices estimating the availability of bird hosts on and surrounding each farm. First, we determined the proportion of nest boxes (e.g. avian density) occupied by Tree swallows and other cavity-nesting species within a 15-km radius around each farm. A nest box was considered occupied when at least one egg was laid during the breeding season. A 15-km radius was used to consider the territory covered by Tree swallows when searching for extra-pair copulations [see Lessard *et al.* (2014) for details]. In addition, we estimated the total farmland bird abundance as well as their species richness and diversity (i.e. Shannon's equitability) based on point counts conducted on each farm during the breeding season of 2004. At least six 5-min point counts were conducted from the central point of each farm during which birds from a pre-established species list (see Table A1) were counted within an unlimited radius.

Statistical analyses

We used mixed logistic regression models to quantify the influence of environmental variables on the haemosporidian infection status of Tree swallows (binomial factor: 0 for uninfected and 1 for infected). For adults and nestlings, we assessed infection status patterns for each parasite genus detected separately (*Plasmodium*

or *Leucocytozoon* species). We also analysed all parasite genera combined for nestlings, given the lower number of individuals tested from a smaller number of farms. All explanatory variables were standardized (zero mean, unit variance) before model selection (see Table A2 and A3). Explanatory variables were selected with a backward selection procedure ($\alpha = 0.05$) and their inclusion/deletion were assessed at each step by using a likelihood ratio test (LRT). Farm and nest-box identities were also included in initial models and tested for significance as random variables with LRTs. Only nest-box identity was significant and kept in nestling models. For individuals with repeated records ($N = 306$), we used only the data obtained at first capture. All variables that were highly correlated ($r > 0.8$) or with high variance inflation factors ($VIF > 3$) were removed from models to avoid collinearity (see Table A4 and A5 for correlation coefficients). All statistical analyses were performed in R 3.1.2 (R Core Team, 2016), with the lme4 package (Bates et al. 2015). The estimation of variance explained (R^2) by the mixed models was obtained with the methods described in Nakagawa and Schielzeth (2013), while the method presented in Nagelkerke (1991) was used for the other models.

RESULTS

Prevalence and lineage diversity

We found a low haemosporidian prevalence in adult Tree swallows for all parasite genera combined between 2012 and 2015 (19% of 1562 individuals; Table 1 and Fig. 2). Prevalence for *Plasmodium* and *Leucocytozoon* species were 7.9% ($N = 125$) and 9.9%, respectively ($N = 155$; Table 1). We found no infection by *Haemoproteus* species (Table 1). A relatively similar overall prevalence was observed for nestlings (17% of 217 individuals) and for each parasite genus (*Plasmodium* species: 9.2%; *Leucocytozoon* species: 5.5%; Table 1). Detection of haemosporidian parasites in nestlings confirmed its transmission on the breeding sites. Overall, 312 lineages were confirmed (from 87% of sequences analysed; Table 2). We found 8 and 2 known lineages for the genus *Plasmodium* and *Leucocytozoon*, respectively (Table 2). CB1 (haplotype of *Leucocytozoon majoris*) and SEIAUR01 (haplotype of *Plasmodium cathemerium*) were the most common known lineages in both adults and nestlings (Table 2). A total of 22 individuals (18 adults and four nestlings) had a mixed infection by parasites either of the same or of a different genus (e.g. *Plasmodium*–*Leucocytozoon*; see Table 3).

Environmental effects

Plasmodium parasite infection status in adult Tree swallows was negatively related to the percentage of anthropic areas within 500 m (Fig. 3A, Table A6). On the other hand, the probability to be infected by *Leucocytozoon* parasites was negatively affected by the mean daily temperature variation (Fig. 3B, Table A6). However, birds located in landscapes with a higher percentage of extensive cultures or of forested areas within 500 m were more likely to be infected by *Leucocytozoon* species (Fig. 3C and D and Table A6). Conducting all analyses without dead individuals and with only the most prevalent lineages (CB1 and SEIAUR01 separately) provided similar results (see Figure A1, A2 and Table A7).

In nestlings, the mean amount of precipitation increased the probability to be infected by all genera combined (Fig. 4 and Table A8). In this analysis, nest-box identity explained 32% of the total variance in the infection status. Analyses of environmental effects conducted separately for *Plasmodium* and *Leucocytozoon* species revealed that none of the variables we considered had a significant effect on haemosporidian prevalence in nestlings (Table A8).

DISCUSSION

Understanding how anthropogenic activities and environmental changes interact to influence parasite prevalence is critical given the increasing number of wild populations affected by human activities. Here, we detected a low prevalence and a moderate lineage diversity of haemosporidian parasites in a declining wild population of Tree swallows. In addition to being the most prevalent lineages found in the adult population, CB1 and SEIAUR01 were also detected in nestlings. Our results thus provide strong evidence that transmission can occur on the breeding grounds of this migratory species. We also showed that the infection status was related to several environmental variables on a fine spatial scale, including the relative amount of anthropic areas and extensive cultures surrounding the birds' nest. However, these environmental characteristics affected haemosporidian prevalence differently depending on the parasites' genus.

Parasite prevalence

The Tree swallow population we studied showed a low prevalence of infection by all haemosporidian parasite genera combined.

Table 1. Summary of individuals analysed and infected by haemosporidian parasites in breeding Tree swallows (*Tachycineta bicolor*) sampled in southern Québec, Canada. Adults were sampled from 2012 to 2015 and nestlings in 2015 only. Prevalence corresponds to the overall proportion of the infected individuals

	Adults					Nestlings 2015	Total
	2012	2013	2014	2015	Total		
<i>Plasmodium</i>	30	24	40	31	125	20	145
<i>Leucocytozoon</i>	37	36	32	50	155	12	167
Single infection	67	60	72	81	280	32	312
Mixed infection	5	2	4	7	18	4	22
Infected individuals	72	62	76	88	298	36	334
Uninfected individuals	264	304	347	349	1264	181	1445
Total	336	366	423	437	1562	217	1779
Prevalence	0.21	0.17	0.18	0.20	0.19	0.17	0.19

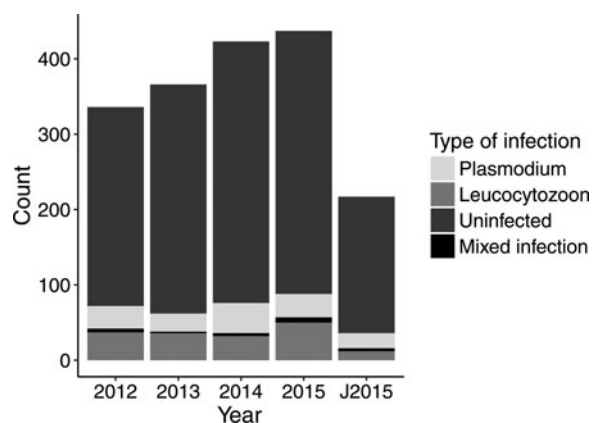


Fig. 2. Annual count of each haemosporidian genus detected in Tree swallow adults (2012–2015) and nestlings (J2015) in southern Québec, Canada.

There is little-published information available to compare the prevalence observed in our study system with that of other Tree swallow populations. Yet, most studies that assessed haemosporidian prevalence in Tree swallows in North America reported low prevalence or absence of infections (Table A9). Similar patterns of relatively low prevalence were also reported in other species of the Hirundinidae family [prevalence range : (0–33%); Oakgrove *et al.* 2014; Medeiros *et al.* 2015; Moens *et al.* 2016; Ellis *et al.* 2017]. This is concordant with the observation that aerial insectivores tend to have low prevalence, likely as a result of the difficulties that insect vectors experience in order to feed on these very mobile birds [prevalence range for other insectivorous passerines: (0–75%); review from Greiner *et al.* 1975; see also: Szymanski and Lovette, 2005]. However, it should be noted that most of the previous studies conducted on Tree swallows in North America were located at lower latitudes and had very low sample sizes ($N < 10$) to assess prevalence with confidence (see Table A9). One exception is the study by Szymanski and Lovette (2005) who used a sampling design comparable with

our study in terms of bird capture technique and parasite detection method (see Table A9). Despite a lower sample size ($N = 89$) and a shorter monitoring period (i.e. 1 year) than in our study, they found a higher global prevalence in Tree swallows breeding near the centre of New York state, USA (48%; see Table A9; Szymanski and Lovette, 2005). Given that local prevalence tends to be stable over time (Fallon *et al.* 2004; Ricklefs *et al.* 2005), these results suggest large-scale spatial variation in haemosporidian prevalence among Tree swallow populations.

We also documented a low prevalence in nestling Tree swallows supporting that haemosporidian parasite transmission can occur during the breeding season at this northern latitude and even in subarctic regions (see also: Oakgrove *et al.* 2014). Since prevalence in nestlings was obtained from a small sample size collected early in the breeding season, our assessment may slightly underestimate the prevalence that could be observed/detected at the end of the season. Indeed, haemosporidian parasites may not be detected in 16 days old nestlings given the presence of a prepatent period [which may vary between 1 and 3 weeks; see Valkiūnas (2005)] in the parasite life cycle. Previous studies also documented avian malaria transmission in North America by the identification of parasites in juvenile migratory birds (Ricklefs *et al.* 2005; Szymanski and Lovette, 2005; Medeiros *et al.* 2015). In particular, the study of Szymanski and Lovette (2005), conducted approximately 500 km south of our study system (Freeville, NY, USA), also detected avian malaria in juvenile Tree swallows. Furthermore, some avian malaria lineages detected in blood meals of trapped mosquitoes in the study of Kimura *et al.* (2010), in Ithaca, NY, USA, were also identified in our Tree swallow population (e.g. PADOM11 and SEIAUR01, see Table 2), suggesting that some common vectors in North America can transmit parasite lineages detected in our study system.

Parasite diversity

The higher diversity of *Plasmodium* lineages detected, compared to *Leucocytozoon*, is concordant with its general low degree of

Table 2. Summary of haemosporidian parasite lineages detected in adult Tree swallows (*Tachycineta bicolor*) from 2012 to 2015 and in nestlings for 2015

Lineages	Adults					Nestlings 2015	Total
	2012	2013	2014	2015	Total		
<i>Plasmodium</i>							
BT7	10	12	13	11	46	0	46
CATUST05	0	0	0	2	2	3	5
GEOTRI09	5	3	6	10	24	0	24
PADOM11	0	0	0	0	0	1	1
SEIAUR01 ^a	6	11	12	11	40	20	60
SGS1 ^a	1	0	0	0	1	0	1
STVAR04	1	0	0	0	1	0	1
WW3	0	0	0	0	0	1	1
Unknown	9	0	12	5	26	0	26
<i>Leucocytozoon</i>							
CB1 ^a	37	32	32	55	156	15	171
TABI09	1	0	1	0	2	0	2
Unknown	7	6	4	1	18	0	18
						Total	356

^aSEIAUR01: *P. cathemerium*; SGS1: *P. relictum*; CB1: *L. majoris*

Table 3. Number of individuals (and lineages) for which each type of mixed infections by haemosporidian parasites were detected in Tree swallow (*Tachycineta bicolor*) for adults from 2012 to 2015 and for nestlings from 2015

Mixed infections	Adults					Nestlings 2015	Total
	2012	2013	2014	2015	Total		
<i>Plasmodium-Plasmodium</i>	0	0	0	1	1	1	2
<i>Plasmodium-Leucocytozoon</i>	2	2	2	6	12	3	15
<i>Leucocytozoon-Leucocytozoon</i>	3	0	2	0	5	0	5
Total	5	2	4	7	18	4	22
Number of lineages	10	4	8	14	36	8	44

host specificity that promotes infection of a large array of migratory birds (Valkiūnas, 2005; Hellgren *et al.* 2007). *Leucocytozoon* species, on the other hand, tend to be more specialized through particular feeding preferences and developmental environmental conditions of their specific vectors, which limit their contact with numerous avian species (Malmqvist *et al.* 2004; Hellgren *et al.* 2008; Svensson-Coelho *et al.* 2016). However, the degree of specificity can vary greatly between lineages of a given genus ranging from a parasite lineage specializing on a single host species to a lineage found in a large diversity of hosts (e.g. CB1 is found in different species from both Europe and North America; see Table A10) (Hellgren, 2005; Medeiros *et al.* 2013). Based on the MalAvi database, all the lineages we amplified were detected in North America, except for SGS1 (haplotype of *Plasmodium relictum*; South America), and detected in Passeriformes, except for STVAR04 (Strigiformes) (Table A10).

The two most abundant lineages in our study system, CB1 and SEIAUR01, were also detected in other Tree swallow populations (e.g. CB1: Murdock, 2005; SEIAUR01: Beadell *et al.* 2006). Six lineages (e.g. STVAR04, TABI09, CATUST05, PADOM11, SGS1 and WW3) were rare in our study system, each represented <2% of all lineages detected. The presence of rare lineages can be the result of sporadic spillovers if competent generalist vectors and permanent reservoir of generalist lineage populations are present in contact zones (Power and Mitchell, 2004; Ricklefs *et al.* 2004; Moens *et al.* 2016). Contact zones on both breeding sites and wintering grounds can act like a transmission corridor between host species (Reullier *et al.* 2006). Lineage competition could also explain the presence of rare lineages that were excluded by more abundant ones in the host organism (Svensson-Coelho *et al.* 2016). Also, more virulent lineages can lead to higher mortality rates of migratory birds before their arrival on breeding

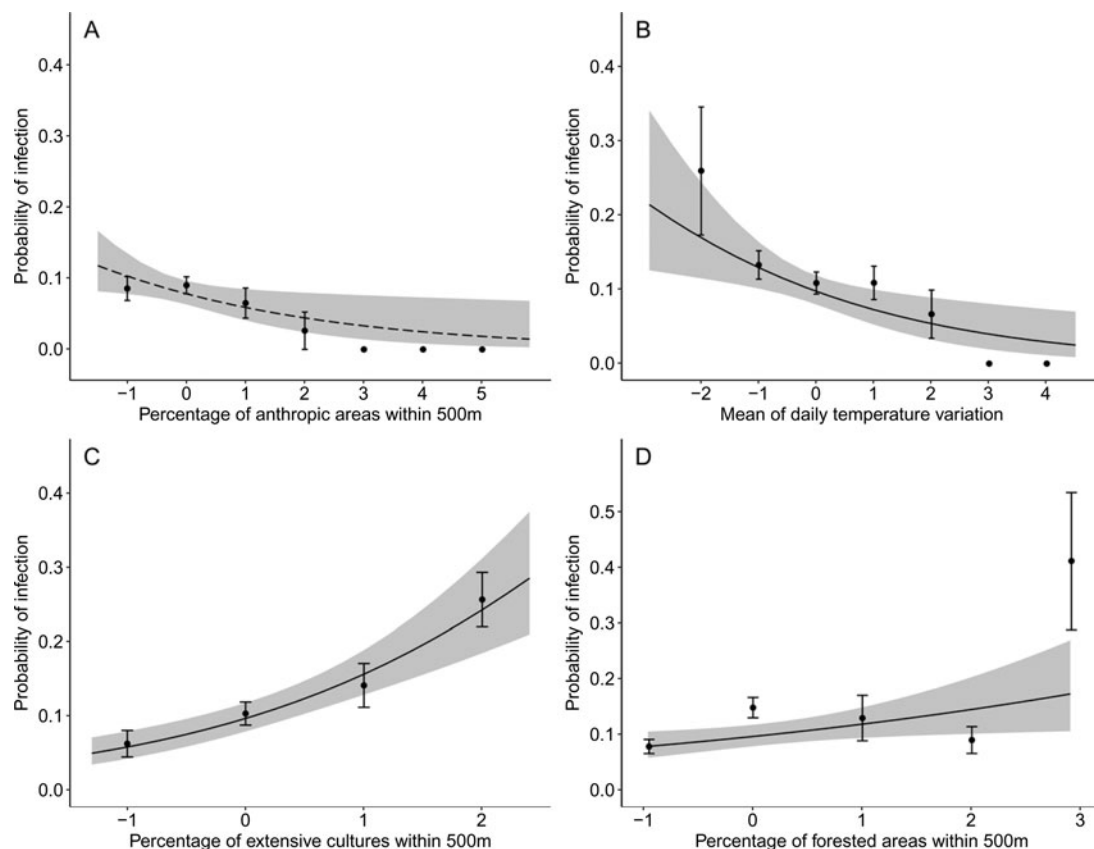


Fig. 3. Relationship between the probability that the birds were infected by *Plasmodium* [final model $R^2 = 0.01$, (A); dashed line] or *Leucocytozoon* parasites [final model $R^2 = 0.09$, (B–D); solid line] separately, modelled by logistic regressions (black dots represent mean \pm s.d.), and standardized environmental variables: (A) percentage of anthropic areas within 500 m; (B) mean of daily temperature variation; (C) percentage of extensive cultures within 500 m; and (D) percentage of forested areas within 500 m in adult Tree swallows, southern Québec, Canada, 2012–2015. Grey areas depict the 95% confidence intervals of predictions.

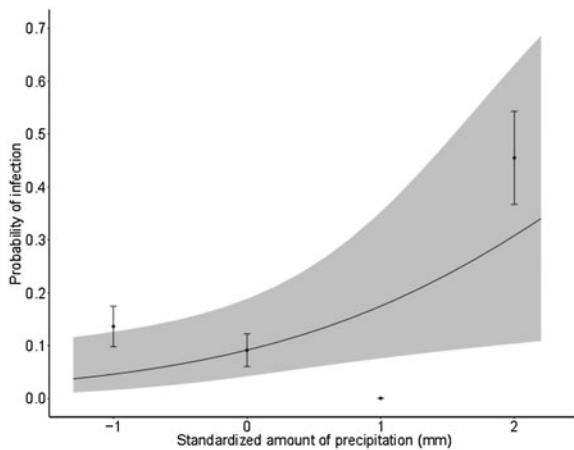


Fig. 4. Relationship between the probability that birds were infected by all haemosporidian genera combined (*Plasmodium* and/or *Leucocytozoon* species), modelled by a mixed logistic regression (black dots represent mean \pm s.d.), and the standardized mean amount of precipitation (mm) in nestling Tree swallows, southern Québec, Canada, 2015. Grey areas depict the 95% confidence intervals of predictions. Nest-box identity was used as a random effect. Final model marginal $R^2 = 0.10$.

sites, resulting in lower prevalence during reproduction (Bensch *et al.* 2007). However, our findings need to be interpreted cautiously given that they are based only on a PCR detection method (Valkiūnas *et al.* 2014). Indeed, haemosporidian parasites detected with PCR methods can represent abortive infections with incomplete development (Valkiūnas *et al.* 2014). Additional evidence such as detection of haemosporidian parasites through microscopy analysis and in blood-sucking insects would be necessary to further support the results we obtained.

We found very few mixed infections in our study system, which was rather surprising given that those are generally common in wild avian populations (Pérez-Tris and Bensch, 2005; Loiseau *et al.* 2010; Palinauskas *et al.* 2011). The low rate of mixed infections can be the result of many types of biological mechanisms such as synergistic interactions and competitions between cohabiting lineages (Bensch *et al.* 2007; Marzal *et al.* 2008; Palinauskas *et al.* 2011; Zehindjiev *et al.* 2012b). However, it is possible that the rate of mixed infections detected can be underestimated due to the PCR methods we used, as preferential amplifications could occur when using primers with greater affinity and/or high intensity of infection is present in some lineages only (Pérez-Tris and Bensch, 2005; Valkiūnas *et al.* 2006; Bensch *et al.* 2007; Martínez *et al.* 2009; Zehindjiev *et al.* 2012b; Synek *et al.* 2013; Bernotienė *et al.* 2016). Thus, complementary blood analysis by microscopy and qPCR would increase confidence in detections of mixed infections, while providing information on the parasitaemia (Richard *et al.* 2002; Valkiūnas *et al.* 2006; Zehindjiev *et al.* 2012b; Bernotienė *et al.* 2016).

Environmental effects

Haemosporidian prevalence was modulated at a fine spatial scale by different environmental factors depending on the parasite genus. This is not surprising given that vector species differ in their optimal environmental requirements during their larval and adult stages of development (Patz *et al.* 2000; Gonzalez-Quevedo *et al.* 2014; Sehgal, 2015). For example, weather factors have been previously identified as major determinants of vector-borne parasite infection risks (Paaijmans *et al.* 2009, 2010; Garamszegi, 2011; Colinet *et al.* 2015). In the population of Tree swallows that we studied, *Leucocytozoon* parasites prevalence was negatively related to daily temperature fluctuations. Mosquitoes undergo spatial and temporal temperature variations

across all of their life stages and those fluctuations may affect these vectors' biology depending on their intensity (Carrington *et al.* 2013; Zhao *et al.* 2014). For instance, Paaijmans *et al.* (2010) associated a decrease in vector and parasite development rates and an increase in mosquito mortality rates with wide fluctuations around the mean high temperatures in tropical regions. These results were subsequently supported by many other studies that also showed low malaria prevalence (*Plasmodium* spp.) under similar climatic conditions (Blanford *et al.* 2013; Paaijmans *et al.* 2013; Estay *et al.* 2014; Colinet *et al.* 2015; Davies *et al.* 2016; Rund *et al.* 2016). However, none of these studies were conducted in temperate regions, and specifically on *Leucocytozoon* species, making it difficult to compare with our study system. Further research is needed to assess if similar effects of temperature fluctuations on prevalence are observed in other populations evolving under temperate climatic conditions.

Anthropic areas were associated with lower prevalence of *Plasmodium* parasites suggesting that parasite transmission patterns were altered by human presence or activities. This supports the findings of previous studies that observed a decrease of haemosporidian infection rates due to anthropogenic perturbations that led to a deterioration of ecosystems and potentially affected the abundance and richness of hosts and vectors (Patz *et al.* 2000; Bradley and Altizer, 2007; Okanga *et al.* 2013; Fourcade *et al.* 2014; Sehgal, 2015). In our study system, higher levels of drainage, generally associated with human activities, may have contributed to the diminution of transmission rates due to the loss of appropriate breeding sites for vectors (Sérandour *et al.* 2007). Habitat perturbations can also alter interspecific competition and feeding habits of vectors implicated in parasite transmission, resulting in decreased prevalence (Bonneaud *et al.* 2009; Chasar *et al.* 2009). Other landscape characteristics, such as the percentage of forested areas and extensive cultures within 500 m, were associated with higher *Leucocytozoon* parasites prevalence. These types of land covers correspond to less perturbed environments and are characteristic of heterogeneous agricultural landscapes with low pesticide inputs (Ghilain and Bélisle, 2008). The vegetation covers of these habitats tend to support greater vector and host abundances, and may thereby facilitate parasite transmission (Mercer *et al.* 2005; Bonneaud *et al.* 2009; Crowder *et al.* 2013; Roiz *et al.* 2015). For example, Bonneaud *et al.* (2009) have previously suggested that high vegetation density may provide protective cover against predation for weak birds infected by *Plasmodium* species when breeding under poor environmental conditions. Finally, the spatial heterogeneity in infection by haemosporidian parasites in our study system supports the idea that transmission occurs locally over breeding sites as suggested by Knowles *et al.* (2014).

Contrary to our expectations, the relative amount of water bodies, as well as avian population density and diversity, did not affect the prevalence of haemosporidian parasites in Tree swallows. The relatively large spatial scales used in our analysis may have prevented the detection of an effect of water bodies on infection status. Indeed, Roiz *et al.* (2015) highlighted the importance of temporary pools in vector development and the difficulty to detect them at large spatial scales. Usually, avian density and diversity are key factors in the transmission of vector-borne parasites (Keasing *et al.* 2006; Fourcade *et al.* 2014; Ellis *et al.* 2017). Dense host populations increase vector–host encounter rates that result in contagion enhancement (Ellis *et al.* 2017). However, an over or under-utilization of hosts by vectors and a lack of parasitic compatibility with some avian species may also limit parasite transmission in specific regions despite a high avian density (Ellis *et al.* 2017).

The amount of precipitation was the only environmental factor that positively affected the probability to be infected by

haemosporidian parasites in nestlings. Rainfall provides the humid environment and temporary pools that are essential for the survival and reproduction of vectors, and subsequently, for haemosporidian transmission (Patz *et al.* 2000; Mercer *et al.* 2005; Galardo *et al.* 2009; Hernández-Lara *et al.* 2017). It should be noted that nest-box identity explained 32% of the total variance in this analysis, suggesting a high amount of variation among nest boxes, which may reflect fine-scale environmental variation that can impinge on the haemosporidian prevalence. The fact that different environmental variables affected juveniles and adults should be interpreted with caution given our limited sampling of nestlings. Further analysis of nestling samples covering the whole study area across multiple years is needed for a better understanding of the mechanisms responsible for haemosporidian parasite transmission at this stage.

Conclusion

Overall, a low prevalence and a moderate lineage diversity of haemosporidian parasites were observed in our Tree swallow population. We also found that climatic and landscape characteristics are important determinants of haemosporidian parasite prevalence and that the effect of these variables differs according to the parasite genus. Interestingly, we showed that some landscapes associated with human activities, such as agriculture and urbanization, can influence the spatial distribution of haemosporidian prevalence. Host–parasite relationships are very complex and our results generate many questions regarding the factors determining the status of haemosporidian infections. A large part of the variation in infection status remained unexplained in our models, suggesting that other variables such as intrinsic factors (e.g. age and sex) are at play in determining haemosporidian prevalence in our study system. Further studies are thus needed before we can better understand the role of vector-borne parasites on the decline of avian populations in temperate environments.

Supplementary material. The supplementary material for this article can be found at <https://doi.org/10.1017/S0031182017002128>

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