J. Parasitol., 100(1), 2014, pp. 59–65

EPIDEMIOLOGY OF *PLASMODIUM RELICTUM* INFECTION IN THE HOUSE SPARROW

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ABSTRACT: In vertebrates, multiple host characteristics and environmental factors are known to influence infectious disease dynamics. Here, we investigated variability in prevalence and parasitemia of *Plasmodium relictum* in the house sparrow (*Passer domesticus*) across a large number of rural and urban populations (n = 16). We found that prevalence was not predicted by any of the host traits investigated (age, sex, body mass, or wing length). However, parasitemia was significantly higher in females when compared to males and in 1-yr-olds as compared to older individuals. Neither prevalence nor parasitemia differed according to habitat type (urban vs. rural). These results suggest that inter-population variation in parasitemia depends on host intrinsic factors whereas variation in prevalence could be due to environmental differences between populations, such as climatic variables that might affect the abundance of vectors. This large-scale study gives us a better understanding of the key factors involved in the epidemiology of avian malaria.

For vector-borne diseases, such as avian malaria and related hemosporidian parasites, multiple biotic and abiotic factors can shape parasite transmission and infectivity (Weatherhead and Bennett, 1991, 1992; Bennett et al., 1995; Merilä et al., 1995; Bosch et al., 1997). Climate, habitat, and altitude have all been reported to influence vector and parasite distribution and abundance (Patz and Olson, 2006; Rogers and Randolph, 2006; Thomson et al., 2006; Wood et al., 2007; Monaghan, 2008; Paaijmans et al., 2010; Bouma et al., 2011; Garamszegi, 2011). In addition, host characteristics, body condition (Kaslow et al., 2008; Palinauskas et al., 2008), and the ability to resist a parasitic infection (Bonneaud et al., 2006; Loiseau et al., 2008) may also be factors in parasite epidemiology.

In birds, host characteristics such as sex, age, and body condition are known to affect the dynamics of infection (Weatherhead and Bennett, 1992; McCurdy et al., 1998). Sex and age are associated with differences in both behavioral and physiological traits, suggesting individuals of a given sex or a given age may be more susceptible to infectious diseases. For example, male and female birds may differ in their susceptibility or exposure to parasites (or both) (McCurdy et al., 1998). The immunosuppressive properties of testosterone are suspected to be involved in male vulnerability to infectious diseases (Poulin, 1996; Schalk and Forbes, 1997; McCurdy et al., 1998; Rolff, 2002; van Oers et al., 2010). However, females usually invest in a large reproductive effort (producing large gametes, incubating eggs, feeding young), and the energetic requirements of reproduction may reduce the amount of resources available for immune defenses. As such, females could be more susceptible to infections when compared to males (Chernin, 1952; Peirce and Marquiss, 1983; McCurdy et al., 1998).

Host age is also an important factor shaping immune function and, hence, parasite resistance. Immune function is typically related with age in a non-linear manner. Young and old individuals are the most likely to suffer from infectious diseases, as young hosts have an inexperienced immune system whereas old hosts usually suffer from a decline in immune surveillance due to senescence (Hudson and Dobson, 1997). One might expect old individuals to being especially vulnerable to infection (due to longer lifetime exposure times and poor immune defenses; Allander and Bennett, 1994; Stjernman et al., 2004; Wood et al., 2007).

Host body condition can also be linked in a complex way to parasite transmission and dynamics (Atkinson et al., 1995, 2000; Karell et al., 2011; Palacios et al., 2012; Shurulinkov et al., 2012). As an example, hosts in poor condition may be predisposed to infectious disease, probably due to a depletion of resources needed for a proper functioning of the immune system (Krasnov et al., 2005). These deficiencies may allow parasite multiplication and transmission (unless overexploitation induces host mortality, thus stopping transmission) and further reduction of body condition (Beldomenico and Begon, 2010). Conversely, hosts in poor body condition may provide fewer resources to parasites, thereby reducing parasite multiplication and transmission (Bedhomme et al., 2004; Tseng, 2006; Tschirren et al., 2007).

Vector-borne parasites are also dependent on environmental conditions, especially in temperate regions where seasonality has a strong influence on survival and development of both parasites and insect vectors. In temperate regions, mosquitoes are emerging during the spring and are active till the end of the summer (Balenghien et al., 2006). Outside the transmission period (i.e., when the mosquitoes are absent from the environment), parasites are maintained in the vertebrate host as chronic infections (Valkiūnas, 2005). In addition to climate conditions, differences in habitat type (e.g., urban or rural) may influence parasite epidemiology (Bradley and Altizer, 2007). Urbanization and industrialization can drastically affect wildlife populations and parasite epidemiology by habitat fragmentation (Lindblade et al., 2000; Overgaard et al., 2003) or by pollutant release (Scheifler et al., 2006; Roux and Marra, 2007; Orlowski et al., 2010). Pollutants released in urbanized habitats might weaken the immune system of the host, making it more susceptible to infectious diseases (Grasman and Scanlon, 1995; Fair and Myers, 2002; Snoeijs et al., 2005; Kenow et al., 2007; Hawley et al., 2009). For example, lead concentrations were found to be positively associated with urbanized habitats and *Plasmodium relictum* prevalence in the

Received 21 December 2012; revised 8 April 2013; accepted 30 April 2013.

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DOI: 10.1645/12-24.1

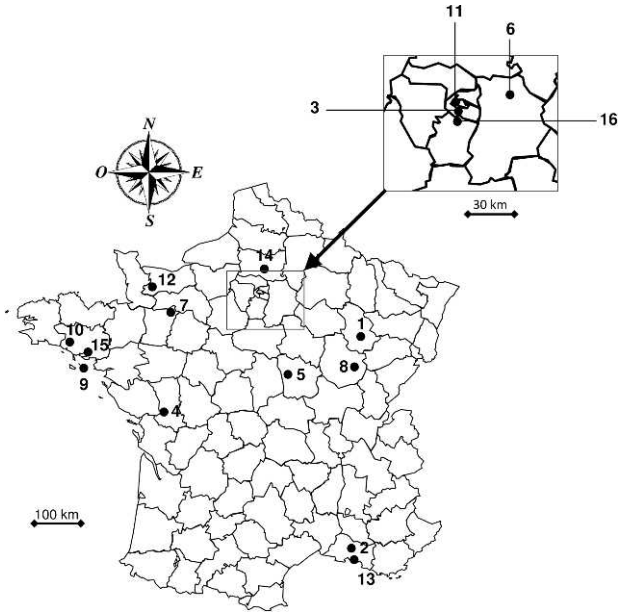

FIGURE 1. Geographic localization of the 16 house sparrow populations: 1 – Anglus, 2 – Arles, 3 – Cachan, 4 – Chizé, 5 – Cosnes-Cours sur Loire, 6 – Crégy les Meaux, 7 – Crennes-sur-Fraubee, 8 – Dijon, 9 – Hoedic, 10 – Languidic, 11 – Paris (Jardin des Plantes), 12 – Rully, 13 – Saintes-Maries-de-la-Mer, 14 – Thieux, 15 – Vannes, and 16 – Wissous. The zoomed region, in the upper right corner, corresponds to the region Ile de France. Scale bar = 100 km for France and 30 km for the region Ile de France.

French house sparrow (Bichet et al., 2013). Important to understand, however, is that these same urbanization factors can negatively affect vector development and abundance, which would lead to a decrease of parasite prevalence in urban habitats (Bentz et al., 2006; Geue and Partecke, 2008; Evans et al., 2009).

Here, we focused on avian malaria parasites and investigated two commonly used disease metrics: prevalence (proportion of infected individuals in a population) and parasitemia (intensity of infection within-host) in association with environmental and host characteristics. Recently, Knowles et al. (2011) investigated fine-scale environmental and host predictors of malaria infection status and parasitemia in blue tits (*Cyanistes caeruleus*). This study suggested that prevalence and parasitemia both increased with host age and that parasitemia was higher in individuals investing more in reproduction. While these findings highlight the ecological complexity of avian malaria in 1 woodland population, this study explores how prevalence and parasitemia vary between multiple populations in different habitats. Thus, we took advantage of our large-scale survey carried out in France of the host–parasite system *Plasmodium relictum*–house sparrow (*Passer domesticus*) (Loiseau et al., 2009, 2011). In 16 urban and rural populations we determined prevalence, quantified parasitemia, and examined the inter-population and inter-individual variations according to host characteristics (age, sex, body mass, and wing length), seasonality, and habitat type (urban or rural).

MATERIALS AND METHODS

Study sites and sampling

We sampled 16 populations of house sparrows (*Passer domesticus*) in France, from 2004 to 2010, during 2 different periods (autumn–winter: October–March and spring–summer: April–September, Fig. 1). Due to field limitations, several populations were not sampled in all seasons or years (Table I). The house sparrow is strongly associated with human development and only occurs within or nearby human activity. Nevertheless, house sparrows can occur in highly urbanized areas as well as in more-rural anthropogenic environments. In order to assess the role played by urbanization on the risk of malaria infection, we scored each of the 16 populations studied here as either urban or rural based on the size of the village–city where sparrows were sampled and on the surrounding habitat. The sample size for each population is summarized in Table I. Each bird was caught using mist-nets and was banded. We measured wing length (±1 mm) and body mass (±0.5 g). Blood samples (~20 µl) were collected by brachial vein puncture and stored in 500 µl of Queen's Lysis Buffer (Seutin et al., 1991) for subsequent molecular analyses.

Each adult bird was sexed based on sexually dimorphic plumage. Each bird was also assigned to 1 of 2 age classes: 1-yr-old (1Y, birds born in the year of sampling) or older than 1-yr-old (>1Y). We obtained the age of individuals for 14 populations and morphological measures for 13 populations (Table I).

Parasite screening

DNA was extracted using standard phenol–chloroform protocol (modified from Hilis et al., 1996). In order to detect the presence of malaria parasites, we used a nested polymerase chain reaction (PCR) (Waldenstrom et al., 2004) to amplify a fragment of the parasite mitochondrial cytochrome *b* gene. We sequenced the positive PCR products and identified lineages using the NCBI nucleotide BLAST search. For this study, we focused on *Plasmodium relictum* (SGS1 and GRW11 lineages), the most predominant parasite species in all populations (Loiseau et al., 2011).

For each positive PCR product, we also performed a relative quantitative PCR (qPCR) to obtain parasitemia following the protocol described in Cellier-Holzem et al. (2010). For each individual we conducted 2 qPCR reactions in the same run: 1 targeted the nuclear 18s rDNA gene of *Plasmodium* (primers 18sPlasm7 [5′-AGC CTG AGA AAT AGC TAC CAC ATC TA-3′], 18sPlasm8 [5′-TGT TAT TTC TTG TCA CTA CCT CTC TTC TTT-3′], and fluorescent probe Plasm Hyb2 [5′-6FAM-CAG CAG GCG CGT AAA TTA CCC AAT TC-BHQ1-3′]) and the other targeted the 18s rDNA gene of birds (primers 18sAv7 [5′-GAA ACT CGC AAT GGC TCA TTA AAT C-3′], 18sAv8 [5′-TAT TAG CTC TAG AAT TAC CAC AGT TAT CCA-3′], and fluorescent probe 18sAv Hyb [5′-VIC-TAT GGT TCC TTT GGT CGC TC-BHQ1-3′]). Parasitemia was calculated as relative quantification values (RQ) as $2^{-(\text{Ct18s}\textit{Plasmodium} - \text{Ct18sBird})}$ using the software SDS 2.2 (Applied Biosystems, Carlsbad, California). Ct represents the number of PCR cycles at which fluorescence is first detected as statistically significant above the baseline, and RQ can be interpreted as the fold-amount of target gene (*Plasmodium* 18s rDNA) with respect to the amount of the reference gene (host 18s rDNA). All qPCR reactions were carried out on an ABI Prism 7900 cycler (Applied Biosystems).

Statistical analyses

We used Spearman correlation coefficients to assess the relationship between mean prevalence and mean parasitemia (log-transformed) across our 16 populations.

We investigated the relationships between patterns of infection and host traits using a combination of multiple regression models. In these statistical models, the dependent variable was either the infection status of sampled individuals (infected or not, hereafter referred to as prevalence, n = 775) or the parasitemia obtained from quantitative PCR (n = 126). The parasitemia variable (log-transformed) was analyzed using linear mixed-effects models (LMM) while the status was examined through generalized linear mixed-effects models (GLMM) assuming binomial distribution of errors and logit link function. Fixed-effect explanatory variables that were used included: the season (binary variable, either spring–summer or autumn–winter), urbanization (binary variable, either urban or rural population), sex (binary), age (binary, either 1Y or >1Y), body mass (g), and wing length (continuous variables). Population and year factors were treated as random variables. We also examined correlations between explanatory variables, which revealed a moderate positive correlation between body weight and wing length (Pearson's $r = 0.20$, $P < 10^{-4}$) and strong variations in wing length among sexes, year, and populations (these 3 variables together explained 50% of wing length variation in linear multiple regression, $P <$

TABLE I. Prevalence (%), parasitemia (log-transformed), body mass (g), and wing length (mm) with sample sizes associated to each of these variables are given for each of the 16 house sparrow populations (U and R refer to urban and rural populations, respectively), in each year and in each season. A–W: autumn–winter, S–S: spring–summer, N1: number of individuals sampled for infection status, N2: number of individuals sampled for parasitemia, N3: number of individuals sampled for body condition.

No.	Population	Year	Season	N1	Prevalence, % (CI)	N2	Parasitemia (±SE)	N3	Body mass, g (±SE)	Wing length, mm (±SE)
1	Anglus (R)	2004	A–W	29	37.93 (20.6–57.7)	11	1.02E–4 (±8.61E–5)	28	29.86 (±0.45)	77.98 (±0.45)
		2005	A–W	27	33.33 (16.4–54.0)	9	1.89E–5 (±1.08E–5)	21	28.05 (±0.41)	78.33 (±0.39)
		Total		56	35.71 (23.2–49.7)	20	6.47E–5 (±4.75E–5)	49	29.04 (±0.33)	78.17 (±0.30)
2	Arles (U)	2005	A–W	27	85.19 (66.1–95.8)	23	7.19E–5 (±2.36E–5)	23	28.54 (±0.37)	77.93 (±0.48)
		2005	S–S	15	66.67 (38.2–88.2)	10	1.30E–3 (±1.31E–3)	14	27.5 (±0.51)	76.11 (±0.89)
		Total		42	78.57 (63.1–89.7)	33	4.84E–4 (±4.18E–4)	44	28.11 (±0.26)	77.14 (±0.43)
3	Cachan (U)	2004	S–S	15	20.00 (4.31–48.1)	3	2.00E–7	15	25.23 (±0.49)	76.33 (±0.63)
		2005	S–S	5	60.00 (14.6–94.8)	3	6.88E–5 (±6.34E–5)	5	26.10 (±0.70)	76.20 (±1.81)
		Total		20	30.00 (11.8–54.3)	6	3.45E–5 (±3.22E–5)	20	25.45 (±0.41)	76.30 (±0.62)
4	Chizé (R)	2005	S–S	48	66.67 (51.4–79.6)	31	8.20E–6 (±3.40E–6)	—	—	—
5	Cosne–Cours sur Loire (R)	2004	A–W	45	48.89 (33.6–64.3)	22	9.45E–5 (±3.37E–5)	45	28.21 (±0.32)	76.25 (±0.14)
		2005	A–W	13	53.84 (25.0–80.7)	7	8.12E–4 (±3.58E–4)	13	27.62 (±0.29)	79.12 (±0.80)
		Total		58	50.00 (36.4–63.5)	29	2.92E–4 (±1.54E–4)	58	28.10 (±0.26)	76.68 (±0.21)
6	Cregy (R)	2004	A–W	21	28.57 (11.2–52.2)	6	6.35E–6 (±5.87E–6)	21	28.76 (±0.39)	78.83 (±0.35)
		2005	A–W	27	44.44 (25.4–64.7)	1	2.00E–7	26	28.94 (±0.30)	79.81 (±0.35)
		2005	S–S	4	25.00 (0.6–80.6)	1	3.29E–06	4	28.13 (±0.22)	79.75 (±0.44)
		2006	A–W	17	47.06 (22.9–72.2)	—	—	17	28.03 (±0.26)	79.03 (±0.40)
		2006	S–S	4	75.00 (19.3–99.3)	—	—	4	27.88 (±0.23)	78.63 (±0.29)
		Total		73	41.10 (29.6–53.3)	8	5.20E–6 (±4.37E–6)	83	28.44 (±0.22)	79.12 (±0.22)
7	Crennes (R)	2004	A–W	7	28.57 (3.7–71.0)	2	8.5E–5 (±7.80E–5)	7	26.29 (±0.56)	76.71 (±0.81)
		2005	A–W	45	33.33 (20.0–49.0)	15	1.08E–4 (±8.40E–5)	17	27.29 (±0.54)	77.57 (±0.56)
		Total		52	32.69 (20.2–47.2)	17	1.05E–4 (±7.41E–5)	26	26.94 (±0.40)	77.23 (±0.44)
8	Dijon (U)	2009	S–S	20	55.00 (31.4–76.9)	11	2.14E–4 (±1.05E–4)	—	—	—
9	Hoedic (R)	2006	S–S	44	6.82 (1.4–18.7)	3	6.90E–5 (±4.07E–5)	—	—	—
		2007	A–W	24	8.33 (1.0–27.0)	2	7.67E–5 (±1.5E–7)	13	28.65 (±0.84)	76 (±0.78)
		2007	S–S	92	4.35 (1.2–10.8)	4	4.58E–4 (±4.15E–4)	—	—	—
		2008	A–W	37	2.70 (0.0–14.1)	1	3.81E–6	24	28.38 (±0.29)	76.65 (±0.37)
		2008	S–S	81	4.94 (1.4–12.3)	3	2.00E–7	14	27.71 (±0.43)	75.86 (±071)
		2009	A–W	53	1.89 (0.0–10.1)	1	1.80E–5	52	28.27 (±0.28)	74.87 (±0.31)
		2009	S–S	143	0.70 (0.00–3.87)	1	5.98E–5	20	27.84 (±0.39)	75.00 (±0.49)
		2010	A–W	11	0.00	—	—	11	30.29 (±0.31)	76.41 (±0.43)
		2010	S–S	200	30.00 (23.7–37.0)	4	6.69E–6 (±6.49E–6)	33	27.63 (±0.29)	74.89 (±0.36)
		Total		685	11.09 (8.8–13.6)	19	1.21E–4 (±8.83E–5)	168	28.17 (±0.14)	75.33 (±0.17)
10	Languidic (R)	2008	A–W	56	16.07 (7.6–28.4)	9	7.66E–5 (±3.53E–5)	56	27.65 (±0.25)	75.01 (±0.29)
11	Paris, Jardin des Plantes (U)	2004	A–W	11	18.18 (2.3–51.8)	2	7.47E–7 (±5.47E–7)	10	26.95 (±0.53)	78.95 (±0.63)
		2004	S–S	27	29.63 (13.7–50.3)	8	8.52E–6 (±6.70E–6)	26	25.58 (±0.39)	78.77 (±0.35)
		2005	A–W	1	100.00	1	2.00E–7	1	27.00	79.00
		2005	S–S	13	38.46 (13.8–68.4)	5	2.22E–7 (±2.54E–7)	5	27.70 (±1.66)	79.40 (±0.75)
		Total		52	30.77 (18.7–45.1)	16	4.55E–6 (±3.53E–6)	42	26.19 (±0.35)	78.89 (±0.27)
12	Rully (R)	2005	A–W	18	50.00 (25.9–74.0)	9	1.37E–4 (±1.17E–4)	18	26.39 (±0.41)	78.11 (±0.53)
		2005	S–S	38	31.58 (17.4–48.6)	10	3.48E–4 (±1.80E–4)	37	26.76 (±0.36)	77.00 (±0.47)
		Total		54	35.19 (22.6–49.4)	19	2.48E–4 (±1.10E–4)	55	26.60 (±0.27)	77.29 (±0.36)
13	Saintes–Marie–de–la–Mer (R)	2007	A–W	20	70.00 (45.6–88.1)	14	1.63E–5 (±7.48E–6)	—	—	—
		2008	A–W	9	77.78 (39.9–97.2)	7	2.34E–5 (±8.35E–6)	—	—	—
		2009	S–S	83	72.29 (61.3–81.6)	36	2.61E–5 (±5.65E–6)	—	—	—
		Total		112	72.32 (61.9–79.5)	57	2.33E–5 (±4.16E–6)	—	—	—
14	Thieux (R)	2004	S–S	45	33.33 (20.0–49.0)	15	1.05E–4 (±8.38E–5)	35	27.86 (±0.35)	78.09 (±0.51)
15	Vannes (U)	2008	A–W	41	24.39 (12.3–40.4)	10	7.39E–5 (±2.17E–5)	40	26.67 (±0.26)	74.86 (±0.30)
16	Wissous (U)	2004	A–W	12	58.33 (27.6–84.9)	7	5.94E–6 (±3.17E–6)	12	26.75 (±0.51)	78.21 (±0.79)
		2004	S–S	27	40.74 (22.3–61.2)	11	3.07E–5 (±1.11E–5)	27	27.06 (±0.58)	78.22 (±0.72)
		Total		39	48.72 (32.3–65.2)	19	2.02E–5 (±7.02E–6)	37	26.96 (±0.30)	78.22 (±0.41)

10^{-4}). The effects of first-order interactions between explanatory variables on prevalence and parasitemia were also investigated. In order to avoid model over-parameterization, we first compared all models, including a single interaction term, to the model without interaction on the basis of the Akaike Information Criterion (AIC). All interaction terms that significantly improved the AIC score (i.e., reducing the AIC by ≥ 2 as compared with the model without interaction) were then included in the final model.

All models presented in the main results are based on GLMM (for status) and LMM (for parasitemia). In order to test the robustness of our conclusions to other modeling frameworks and to compute accurately the

TABLE II. AIC values for the mixed models conducted for prevalence and parasitemia. Missing values (–) indicate that the model did not converge. Interactions that improved model fit (reducing the AIC by ≥2 as compared to the model without interaction) were included in the final model.

Dependant variable	Prevalence	Parasitemia
Sample size	775	126
No interaction	454.1317	350.9338
season×sex	453.3917	349.8699
season×age	455.8444	349.2505
season×weight	455.9944	353.7176
season×wing length	454.3304	348.1664
season×population	456.1317	352.9338
sex×age	455.5478	346.1485
sex×weight	456.1135	353.9513
sex×wing length	455.0481	354.2791
sex×population	456.1317	352.9338
age×weight	456.1036	352.9289
age×wing length	454.9425	352.9304
age×population	–	–
weight×wing length	466.3952	357.5665
weight×population	–	–
wing length ×population	–	–
year×season	456.1317	352.3697
year×sex	456.1041	352.9274
year×age	–	–
year×weight	–	–
year×wing length	–	–
year×population	456.1317	352.9338
urbanization×season	456.1314	351.0125
urbanization×sex	455.9468	350.9587
urbanization×age	455.9468	350.9587
urbanization×weight	453.7248	353.8833
urbanization×wing length	455.8103	353.6134
urbanization×population	–	–
urbanization×year	–	–
Final model	454.1317	342.5857

proportions of independent variance explained by each explanatory variable, we also performed hierarchical partitioning (HP; Chevan and Sutherland, 1991). HP uses all models in a regression hierarchy to distinguish those variables that have high independent correlations with the dependent variable (Mac Nally, 2002). Results are expressed in terms of percentage of total independent effect of each explanatory variable on the dependent variable (R^2). A randomization test (1,000 iterations) was performed for testing the significance of each explanatory variable in each analysis (Walsh and Mac Nally, 2003). Interactions were not considered in HP models.

All statistical analyses were conducted using the R 2.10.1 framework (R Development Core Team, 2009).

RESULTS

Overall, *Plasmodium* prevalence varied from 11 to 79% (Table I). Prevalence in urban habitats (35.41%) was not significantly different from prevalence in rural habitats (39.42%), as the urbanization variable and the associated interactions did not improve the model fit (Table II).

Prevalence was 18.97% in 1Y birds and 31.73% for older individuals (>1Y). Twenty-two percent of females were infected vs. 27% of males. In the GLMM model with no interactions fitted, no fixed-effect explanatory variable was significantly correlated with prevalence but HP models indicate significant differences in prevalence variation among populations ($Z = 7.7$, $P < 10^{-4}$), with population explaining 11.3% of the variance in prevalence. No interaction terms significantly improved model fit (Table II).

TABLE III. Linear mixed-effects model on parasitemia (log-transformed). Fixed-effect variables were the season (either spring–summer or autumn–winter), sex, age (either 1Y or >1Y), urbanization (either urban or rural), body mass, and wing length (continuous variables). The population and year factors were treated as random variables. SE: standard error of the estimate; *t*: Student statistics.

	Estimate	SE	*t*	*P*-value
season	34.477	12.488	2.761	0.006
sex	−4.099	1.323	−3.098	0.002
age	−2.183	0.927	−2.355	0.019
weight	−0.125	0.114	−1.100	0.271
wing length	0.290	0.137	2.120	0.034
urbanization	0.510	0.606	0.842	0.400
sex × age	3.265	1.416	2.306	0.021
season × wing length	−0.446	0.161	−2.772	0.006

Parasitemia varied from $4.55.10^{-6} \pm 3.53.10^{-6}$ to $4.84.10^{-4} \pm 4.18.10^{-4}$ (Table I) and did not differ between urban ($1.39.10^{-4} \pm 7.57.10^{-5}$) and rural habitats ($1.04.10^{-4} \pm 3.06.10^{-5}$; $P = 0.4$, Table III).

Among infected individuals, host characteristics had significant effects on parasitemia. Although no fixed-effect predictor was significantly correlated with parasitemia in mixed-effects models without interactions, the sex × age and the season × wing length interaction terms improved model fit (Table II) and revealed sex and age variations in parasitemia (Table III). Overall the model indicated that (a) parasitemia was lower for males as compared to females, (b) 1-yr-old females tended to have higher parasitemia than did older females, (c) 1-yr-old males tended to have lower parasitemia than did older males (Table III; Fig. 2), and (d) the wing length was negatively correlated with parasitemia in the spring–summer season and positively correlated in the autumn–winter season (Table III).

Among the 16 populations, there was no statistically significant correlation between prevalence and parasitemia ($R_S = 0.06$, $P = 0.84$).

DISCUSSION

Parasite prevalence and parasitemia are known to vary in space and time, and several abiotic or biotic (or both) parameters are likely responsible for this variation (LaPointe et al., 2010). To our knowledge, few studies have measured parasitemia and prevalence of the same parasite species across populations of the same host. Here we show that, in a large number of natural house sparrow populations, host characteristics, age, sex, and wing length were correlated with *Plasmodium* parasitemia but not with prevalence.

We found differences in parasitemia between sexes when interacting with age, with females generally suffering higher parasitemia than did males. Previous work on sexual differences in parasite infection has largely shown a higher susceptibility of males because testosterone and corticosterone produced by males during the reproductive season can substantially weaken the immune response (Poulin, 1996; Schalk and Forbes, 1997;

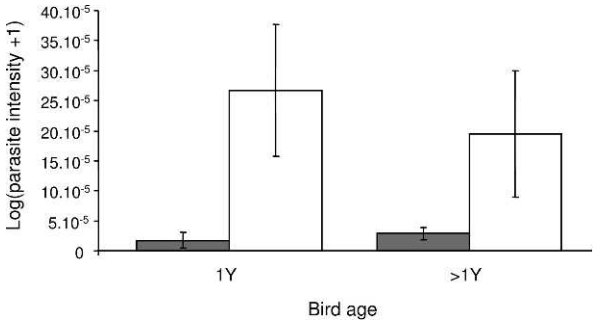

FIGURE 2. Parasitemia (log+1) of male (grey boxes) and female (empty boxes) house sparrows aged 1 yr (1Y) or older (>1Y). Means ± SE are reported.

McCurdy et al., 1998; Rolff, 2002; van Oers et al., 2010). Alternatively, differences in parasitemia between males and females might reflect different ethologies between sexes. For instance, females might be an easy target for vectors during the extended time spent in the nest to incubate eggs and brood hatchlings (Chernin, 1952; Peirce and Marquiss, 1983; Korpimaki et al., 1993; Norris et al., 1994). However, in addition to higher parasitemia, this should also result in a higher prevalence in females than in males. Our finding of statistically similar prevalence between sexes does not support a differential exposure hypothesis. Tentatively, one might speculate that the cost of reproduction is higher in females than in males. This differential reproductive effort could potentially make female immune response less competent when facing a malaria infection (Richner et al., 1995; Williams, 2005), but this idea remains to be tested.

House sparrow age also had an effect on *Plasmodium* parasitemia, with 1-yr-old birds generally harboring a higher parasitemia than did older individuals, although a quantitative difference between individuals of different ages was sex-dependent. This is a finding that has been reported in a variety of systems (Graves et al., 1988; Gregory et al., 1992; Allander and Bennett, 1994; Merilä et al., 1995; Dawson and Bortolotti, 2000; Sol et al., 2000, 2003; Amo et al., 2005; Hasselquist et al., 2007; Syafruddin et al., 2009). As for the effect of sex, the observed age effect could be due to a differential exposure to vectors or, most likely, due to the development of a competent immune response as long as birds become exposed and re-exposed to the parasite (Graczyk et al., 1994; Hudson and Dobson, 1997; Atkinson et al., 2001; Sol et al., 2003). This has been previously demonstrated in immunologically naïve canaries that were experimentally infected with *Plasmodium relictum* (SGS1 lineage). These hosts suffer from higher parasitemia compared to individuals that have already been exposed to the parasite (Cellier-Holzem et al., 2010).

Parasitemia was associated with wing length in a complex way. Wing length was positively associated with parasitemia in fall–winter and negatively in spring–summer. This pattern might reflect the cost of molt, which occurs at the end of summer in the house sparrow. Individuals that molt longer feathers might be more prone to harbor larger number of parasites because of the energetic requirements of molting. This also remains a speculative argument that would require further investigation.

Surprisingly, we did not find strong support for seasonality variation influencing either prevalence or parasitemia in house sparrows. This result suggests that although transmission only occurs during the season when mosquitoes are available, parasites manage to maintain a year-round, stable prevalence and parasitemia in host populations.

Finally, we did not find prevalence and parasitemia differences between urbanized and rural habitats. While in some cases animals living in urban habitats may be protected from infectious diseases, there have been reports of increased transmission of pathogens in urban areas (Bradley and Altizer, 2007). The lack of difference in prevalence and parasitemia between rural and urban populations might reflect similar vector abundance across the environmental gradient considered in this study. A survey of vectors in both habitats would help understand the impacts of urbanization in avian malaria epidemiology.

To conclude, our large-scale survey of house sparrow populations showed that prevalence and parasitemia are independent traits that must be studied in parallel. In the case of avian malaria, parasitemia seems to depend on host traits, such as age and sex, while prevalence may depend more on abiotic factors (Loiseau et al., 2013) that are most likely driving vector abundance (Wood et al., 2007; Fokidis et al., 2008; Geue and Partecke, 2008; Evans et al., 2009).

ACKNOWLEDGMENTS

We thank all the volunteers from the National Museum of Natural History of Paris and from the University of Bourgogne for their help in the field and for providing samples: F. Baroteau, J. Besnault, J. Birard, M. Brenier, L. Brucy, P. Bulens, E. Cellier-Holzem, P. Cannesson, G. Chaussi, B. Faivre, P. Fiquet, C. Girard, D. Lavogiez, J. Marion, F. Martayan, G. Massez, P. Ollivier, V. Ternois, and M. Zucca. We thank also Anne Besson and Ryan J. Harrigan for their comments. Financial support was provided by the Région Ile de France to C.L., R.J., and G.S. and by the Région Bourgogne to G.S., and by grants from OTKA (PD76862), NSF (IOS-1145625) and an Eötvös State Grants (MÖB) to A.Z.L.

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