Host-parasite interaction explains variation in prevalence of avian haemosporidians at the community level.

L. Garcia-Longoria1*, A. Marzal1, F. de Lope1 and L. Z. Garamszegi2

[1] Departamento de Anatomía, Biología Celular y Zoología, Universidad de Extremadura, E-506071 Badajoz (Spain).

[2] Department of Evolutionary Ecology, Estación Biológica de Doñana-CSIC, E-41092 Seville, Spain

*Corresponding author: e-mail: luzlongoria@unex.es

Tel.: 0034924289412

Fax: 0034924289417

ABSTRACT

Parasites are a selective force that shape host community structure and dynamics, but host communities can also influence parasitism. Understanding the dual nature from host-parasite interactions can be facilitated by quantifying the variation in parasite prevalence (i.e. the proportion of infected host individuals in a population) among host species and then comparing that variation to other ecological factors that are known to also shape host communities. Avian haemosporidian parasites (e.g. *Plasmodium* and *Haemoproteus*) are abundant and widespread representing an excellent model for the study of host-parasite interactions. Several geographic and environmental factors have been suggested to determine prevalence of avian haemosporidians in bird communities. However, much remains to be known regarding whether host and parasite traits, represented by phylogenetic distances among species and degree of specialization in host-parasite relationships, can influence parasite prevalence. The aims of this study were to analyze factors affecting prevalence in a bird community and to test whether the degree of parasite specialization on their hosts is determined by host traits. Our statistical analyses suggest that prevalence is mainly determined by the interaction between host species and parasite lineages where tolerance and/or susceptibility to parasites plays an essential role. Additionally, we found that although some of the parasite lineages infected a low number of bird species, the species they infected were distantly related and therefore the parasites themselves should not be considered typical host specialists. Prevalence was higher for generalist than for specialist parasites in some, but not all, host species. These results suggest that prevalence mainly results from the interaction between host immune defences and parasite exploitation strategies wherein the result of an association between particular parasite lineages and particular host species is idiosyncratic.

41 Keywords: bird community, *Haemoproteus*, host-parasite interaction, *Plasmodium*,
42 prevalence.

INTRODUCTION

Parasites have been suggested as a selective force since they might shape host community dynamics [1], alter interspecific competition and influence energy flow [2]. An essential trait in studies of host-parasite interactions is prevalence (i.e. the proportion of individuals infected by a parasite or pathogen in a population at one point in time) [3]. The importance of prevalence is reflected in the amount of studies showing how major ecological factors shaped its intra- and interspecific variation [4,5]. In bird communities, geographic areas, environmental issues or host species/population have been suggested as factors determining prevalence [6]. However, more studies are still needed in order to understand mainly factors affecting parasite prevalence at the community level.

Avian *Plasmodium* and *Haemoproteus* spp. represent a well-studied host-parasite system. These parasites are vector-transmitted organisms that can cause host mortality or morbidity during the acute phase of infection impacting the life histories of their hosts [7]. Their life-cycle is complex, involving sexual stages in their dipteran vectors and asexual stages in their vertebrate hosts [7]. Prevalence of these parasites may be affected by different factors concerning the vector, hosts and the parasites themselves [7]. Some authors have focused their attention on the possibility that host traits might determine prevalence of these parasites [8–10]. For instance, blood parasite susceptibility of both bird individual [11] and bird species [12,13] have been recently suggested as factors affecting prevalence. In this sense, some bird species may have developed both tolerance and resistance mechanisms [14] underlying the importance of bird species in prevalence studies.

In addition to characteristics of hosts, characteristics of parasites themselves can affect their prevalence in wild communities. Haemosporidians parasites present high

68 plasticity and versatility reflected in the number of parasite lineages found among bird
69 species. Thus, within haemosporidian parasites there are more than 3000 parasite
70 lineages [15] infecting more that 1500 bird species. Each of these lineages may have
71 diverse virulence [16] which could result in an array of different negative effects inside
72 the hosts. The plasticity that malaria lineages may have [17,18] allow these parasites to
73 fully exploit hosts reacting even to changes in the physiological state of the host or the
74 environment [19]. Thus, prevalence displayed by parasite lineage in a determined host
75 species or individual is simply the interaction between host immune defences
76 (susceptibility or tolerance) and parasite strategy (virulence). Additionally, malaria
77 parasites must find the correct host in order to complete its life cycle and achieve a high
78 transmission rate [3,20]. To find the precise host is crucial for specialist parasites that
79 need a particular bird species unlike generalist parasites that infect a wider range of bird
80 species [21]. However, and despite the large number of studies focused on
81 haemosporidian parasites, there are a scarce number of them dealing with factors
82 affecting prevalence in wild communities from the perspectives of both the host and
83 parasites.

84 *Plasmodium* and *Haemoproteus* spp. might be detected in several host species
85 that can be distantly or closely related to each other [22–24]. Mechanisms allowing
86 parasite to switch host is a central issue nowadays in avian malaria studies [25,26]. Host
87 switching mechanism is a strategy that malaria parasites may use, in order to infect
88 larger number of bird species [27,28], where closely parasite lineages may be detected
89 in bird species with similar immune defences [29–31]. Thus, parasite lineages infecting
90 close related bird species may exhibit the same exploitation strategy for avoiding host
91 defences [32]. Conversely, more generalist parasites would be able to infect a broad
92 range of bird species that might be distantly related [33]. Analysing phylogenetic

93 distances between bird species sharing the same parasite lineages become essential in
94 community studies in order to determine parasite strategies and how specialist and
95 generalist parasites exploit different host species. However, little is know about how
96 haemosporidian parasite lineages infect bird host species depending on the host
97 phylogenetic distance.

98 The fact that haemosporidian parasites need a vector for completing their cycle
99 life extends the number of elements affecting prevalence of these parasites. Although
100 some studies have shown limited or no relationship between climate and prevalence
101 [34], some have showed that vector populations might increase their number due to
102 climate change and, therefore, vector-borne infectious diseases may increase every year
103 [35]. Vector availability might also change among seasons, thus, during spring the
104 amount of vectors in the wild increase [36] raising the probabilities to become infected
105 [37]. During this season the probability of becoming infected increases significantly not
106 only because of vector availability but also because of the secretion of sexual hormones
107 that may alter immune system and, therefore, facilitate the entrance of blood parasite in
108 the vertebrate host [38]. These studies emphasize the importance of including both year
109 and season of sampling for studies focused in prevalence of haemosporidians
110 prevalence when analysing communities.

111 Taken as a whole, studies focused on prevalence at the community level have
112 analysed certain factors affecting the presence of prevalence separately. However, to
113 date there is no study dealing with the main factors (i.e. host species, parasite lineage
114 and vector conditions) that might affect the presence of haemosporidians parasites or
115 with the parasite strategy depending on the host phylogenetic distance. Hence, the aims
116 of this study are (i) to analyse factors affecting prevalence of haemosporidian parasites
117 in a wild bird community such as phylogenetic distances, parasite phylogeny, host ID,

parasite lineage or seasonal effects and (ii) compare the phylogenetic distances among bird species infected with the same parasite lineage in order to determine whether the parasite infects phylogenetic close host species. For this purpose, factors such as year, day of sampling, bird species, parasite lineage, season of sampling, host-parasite interaction and phylogenetic relationships among avian hosts were included in the analyses and related with prevalence of haemosporidian parasites.

MATERIAL AND METHODS

Data collection and database

The study was carried out from February to October during a 9-year period (2002-2010) in the surroundings of Badajoz (SW Spain) (38°52'N, 705'W). Birds were collected always during the sunrise and during the early hours of the morning. Using a mist-net system, we captured a total of 815 individuals from 21 bird species belonging to nine different families. We obtained one microcapillary of blood (70µl) from the brachial vein of each individual and stored it in 500 µl of 96% ethanol until analysis.

Methods were approved by Institutional Commision of Bioethics of Univ. of Extremadura (CBUE 49/2011)

Prevalence and genetic detection of parasite lineages

DNA from the avian blood samples were extracted in the laboratory using a standard chloroform / isoamylalcohol method [39]. Diluted genomic DNA (25 ng/µL) was used as a template in a polymerase chain reaction (PCR) assay for detection of the parasites using the nested-PCR protocols described [40]. The amplification was evaluated by running 2.5 µL of the final PCR on a 2% agarose gel. All PCR experiments contained one negative control for every eight samples. In the very few cases of negative controls

143 showing signs of amplification (never more than faint bands in agarose gels), the whole
144 PCR-batch was run again to make sure that all positives were true. All samples with
145 positive amplification were sequenced directly using procedures described [41]. The
146 obtained sequences of 478 bp of the cyt b were edited, aligned and compared in a
147 sequence identity matrix using the program BioEdit [42]. Parasites with sequences
148 differing by one nucleotide substitution were considered to represent evolutionary
149 independent lineages [43]. Five new sequences were deposited in GenBank under the
150 accession numbers JQ749720 – JQ749724.
151
152 **Phylogenetic analyses and statistical procedures**
153 For our statistical models (see below) we created two different phylogenetic trees
154 (supporting information Figs S3 and S4) to control for the common descent of different
155 parties: one for the host of bird species and one for the parasite lineages. The first one
156 relied on the 1000 trees generated by the birdtree.org website [44], from which a
157 consensus tree was created for the sampled 21 species by using Geneious v5.4 [45]. The
158 second tree relied the sequences of the parasite lineages [15] identified in this study.
159 Then, we created a phylogenetic tree using MrBayes 3.1 [46,47]. We used jModelTest
160 0.1 [48] in order to determine which parasite tree offered the best fit to our data. The
161 burn-in was identified through Tracer 1.2.2 [49]. We sampled 10 million generations at
162 intervals of 1000. Finally we analysed the files generated by Bayesian MCMC runs by
163 MrBayes in Tracer with the objective of confirm whether the parasite tree generated
164 was the most adequate to our analyses.
165 These phylogenetic trees were entered in the subsequent statistical models
166 sequentially to evaluate the same list of predictors (first, we run the model using the
167 bird phylogenetic tree and then we run the same model but we used the parasite

168 phylogenetic tree). The general strategy for our modelling was to incorporate different
169 factors that might be related to: (i) vector effects (breeding season or non-breeding
170 season, year and date of sampling), (ii) host effects (host species, phylogenetic
171 relatedness), (iii) parasite effects (lineage identity and parasite phylogenetic
172 relatedness), and (iv) host-parasite interaction (the combination of bird species and
173 parasite lineage). Given that our parasite screening method allowed us to detect
174 prevalence (yes or no) at the individual level for each parasite lineage screened, we
175 could also incorporate effects due to host individual identity. We used the Bayesian
176 framework for generalized mixed models (GLMM) incorporating Markov chain Monte
177 Carlo (MCMC) estimation available in the package 'MCMCglmm' [50]. Because
178 dependent variable (a given parasite lineage detected or not in a given individual of a
179 given species) has a binomial distribution, we adopted the "categorical" family of
180 distribution. The fixed variables we considered were the date of sampling and the
181 breeding status of individuals (breeding or not), as these factors may affect infection
182 status due to mosquito abundance/activity and susceptibility of hosts during the
183 demanding chick-feeding period [7]. The year of sampling, parasite lineage, bird
184 individual identity, host species and phylogenetic relationships (parasite or host) were
185 used as random factors. Additionally, we also included the interaction between host
186 species and parasite lineage as a random factor [11,51]. Plots were made with the R
187 package ggplot2 (v. 2.1.0) [52]. All the statistical analyses were carried out with the
188 program R v.1.1.383 [53].

189 We used uninformative priors with a low degree of belief in all parameters. The
190 model was run for 130000 iterations preceded by a burn-in of 30000 iterations, and
191 sampling every 100 iterations to avoid autocorrelation. We evaluated model
192 convergence visually by plotting the chains and checking that they had mixed properly

and by plotting the autocorrelation (supporting information Figs S1 and S2). Once both model were finished we rescaled our parameters by estimating the marginal parameter modes using Kernel Density Estimation [50].

We examined phylogenetic relatedness of bird species infected by the same lineages, and determined if species with non-zero prevalence are more closely related to each other than could be expected by chance. The significance of this metric was tested by comparison to a null distribution derived from 999 random permutations among the tips of the phylogenetic tree followed by calculation of the main pairwise distance (MPD). Calculations were carried out with the ses.mpd function of the R package Picante [54]. When running a main pairwise distance calculation different value comes out. Thus, MPDobs = observed mean pair-wise phylogenetic distance between all species pairs infected with the parasite. The mean and standard deviation of MPD in the null distribution were obtained by randomization of species in the phylogenetic distance matrix (taxa.labels method in Picante). Z = (MPDobs – mean MPD of the null distribution)/SD of the null distribution. Negative values of Z indicate greater phylogenetic homogeneity (clustering). P is the probability of drawing an MPD from the null distribution at least as extreme as MPDobs, based on 999 randomizations.

RESULTS

Total prevalence and lineages detected

We determined the presence of 26 parasite lineages belonging to the genera *Haemoproteus* (N = 13) and *Plasmodium* (N = 13) in the analysed host species (Table 1). We found that 63.80% of total individuals and 81 % of species were infected by haemosporidian parasites. We detected five new parasite lineages that had not been previously described (Table 2).

Table 1. Prevalence and genetic identity of haemosporidian parasite lineages in Passeriform host species analyzed in this study. and parasite lineage found in each host species.

FAMILY	SPECIES	PREVALENCE (%) (N infected / N tested)	PARASITES LINEAGES
Aegithalidae	*Aegithalos caudatus*	0 (0 / 4)	
Certhiidae	*Certhia brachydactyla*	0 (0 / 8)	
Fringillidae	*Carduelis cannabina*	20 (1 / 5)	SGS1
	Carduelis carduelis	18.75 (3 / 16)	CARDUEL1,CARDUEL2
	Serinus serinus	6.67 (1 / 15)	CCF2
	Carduelis chloris	16.67 (1 / 6)	CARDUEL3
	Fringilla coelebs	10.0 (1 / 10)	SGS1
Hirundinidae	*Delichon urbicum*	68.55 (266 / 388)	DURB6, GRW2, GRW4, GRW9, SGS1, RTSR1, DELURB1, DELURB2, DELURB3, DELURB5
	Hirundo rustica	17.35 (30 / 168)	DELURB2, DELURB5, GRW9
	Riparia riparia	14.28 (4 / 28)	CCF2
Paridae	*Cyanistes caeruleus*	33.33 (2 / 6)	SGS1
	Parus major	25 (2 / 8)	HIPOL1, SGS1
Passeridae	*Passer domesticus*	64.57 (177 / 275)	COLL1, GRW11, PADOM2, PADOM5, PADOM1, SGS1, PADOM8, PADOM22, PAHIS1, GRW11
	Passer hispaniolensis	67.74 (21 / 31)	PADOM5, SGS1, PADOM1
Sturnidae	*Sturnus unicolor*	0 (0 / 5)	
Sylviidae	*Phylloscopus collybita*	7.14 (1 / 14)	HIPOL1
	Sylvia atricapilla	28.57 (2 / 7)	SGS1
	Sylvia melanocephala	12.12 (4 / 33)	SGS1, SYMEL1
Turdidae	*Erithacus rubecula*	16.67 (1 / 6)	ROBIN1
	Luscinia megarhynchos	0 (0 / 15)	
	Turdus merula	30 (3 / 10)	SYAT5, TURDUS3
TOTAL INDIVIDUAL ANALYZED		Mean = 63.80 (520 / 815)	

221 **Table 2.** List of parasite lineages found in the current study. New detected parasite
222 lineages are marked in bold.

Parasite lineage	Morphological species	GenBank acc. Number	Number of host species infected
COLL 1	*Plasmodium sp.*	AY831747	1
DELURB1	*Haemoproteus hirundinis*	EU154343	1
DELURB2	*Haemoproteus sp.*	EU154344	2
DELURB3	*Haemoproteus sp.*	EU154345	1
DELURB5	*Plasmodium sp.*	EU154347	2
DURB6	*Plasmodium sp.*	EU219392	1
GRW11	*Plasmodium relictum*	AY831748	1
GRW2	*Plasmodium ashfordi*	AF254962	1
GRW4	*Plasmodium relictum*	AF254975	1
GRW9	*Plasmodium sp.*	EU810681	2
HIPOL1	*Haemoproteus sp.*	DQ000324	2
ROBIN1	*Haemoproteus attenautus*	AY393807	1
CCF2	*Haemoproteus sp.*	AF495551	2
CARDUEL1	***Haemoproteus sp.***	**JQ749720**	**1**
CARDUEL2	***Haemoproteus sp.***	**JQ749721**	**1**
CARDUEL3	***Haemoproteus sp.***	**JQ749722**	**1**
SYMEL1	***Haemoproteus sp.***	**JQ749723**	**1**
TURDUS3	***Plasmodium sp.***	**JQ749724**	**1**
PADOM01	*Plasmodium sp.*	DQ058611	2
PADOM02	*Plasmodium sp.*	DQ058612	1
PADOM05	*Haemoproteus passeris*	HM146898	2
PADOM08	*Plasmodium sp.*	GU065648	1
PADOM22	*Haemoproteus sp.*	GU065650	1
PAHIS1	*Haemoproteus sp.*	GU065651	1
SGS1	*Plasmodium relictum*	AF495571	9
RTSR1	*Plasmodium sp.*	AF495568	1

223

224 **Factors affecting prevalence**

225 Our results suggest that bird defences and parasite virulence (i.e. host-parasite
226 interaction) affect infection status stronger than the rest of the variables analysed (Table
227 3) where this interaction might be the most important factor determining prevalence.
228 Additionally, two more factors appeared to affect variance in prevalence, although on a
229 lesser scale. Thus, our models suggested that breeding season and parasite lineage might
230 affect haemosporidian prevalence.

231 **Table 3.** Variance of prevalence in relation to fix and random factors. Results of both models are showed. Bolded values represent factors that
232 were statistically significant.

	Bird				Parasite			
	Mean	Lower/upper 95% CI	*p*MCMC	Rescaled mean	Mean	Lower/upper 95% CI	*p*MCMC	Rescaled mean
Fixed factors								
Day	-4.055e-03	-8.653-03 / 7.416e-04	0.108	-0.004	-3.503e-03	-8.545e-03 / 7.867e-04	0.152	-0.035
Breeding season	**1.825e+03**	**-3.292e-01 / 4.2623+00**	**0.092**	**2.071**	**1.7373**	**-1.212e-01 / 3.842**	**0.074**	**1.775**
Random factors								
Year	1.616	0.243 / 4.364		0.415	1.367	0.181 / 3.374		0.578
Lineage	**2.208**	**0.0002 / 4.897**		**1.335**	**1.436**	**0.0002 / 3.600**		**1.009**
Individual	0.006	0.0001 / 0.018		0.005	0.007	0.0002 / 0.021		0.007
Species	0.126	0.0003 / 0.567		0.007	0.166	0.0001 / 0.845		0.006
Linage* Species	**13.26**	**8.318 / 21.97**		**7.007**	**11.030**	**6.785 / 15.590**		**7.076**
Phylo	0.008	0.0002 / 0.028		0.007	0.1629	0.0002 / 0.747		0003

233

234

235 **Infected bird species and phylogenetic distances**

236 The random effects for parasite lineage shows that there are considerable differences
237 among parasite lineages in their host exploitation strategies. We found that the most
238 prevalent parasite lineage was *Plasmodium relictum* SGS1 as it was detected in 9 out 21
239 host species. However, the prevalence in particular host species was quite variable (see
240 Fig. 1). In contrast, the other lineages seem to be more specialists, at the present study,
241 as they were detected in fewer species.

242

243 **Figure 1:** Prevalence of each parasite lineage in every host species found in the
244 community. Bird species with no infection are not present in the figure. Lineages
245 infecting more than one bird species have been colored marked while parasite lineages
246 infected only one host species remain in grey. Noted that depending on the sample size
247 the dot showing the prevalence change its magnitude.

248

249

250 We detected 8 different lineages infecting, at least, two host species (Fig. 1).
251 Thus, we analysed the phylogenetic distance between the hosts species infected by one
252 relative to the average phylogenetic distance between two species on the entire tree. Our
253 results showed that *Haemoproteus* spp. CCF2 lineage, *Plasmodium* spp. HIPOL1,
254 GRW9 and *P. relictum* SGS1 infected non-closely related bird species. Moreover, we
255 detected that *Haemoproteus passeris* PADOM05 lineage; *Haemoproteus* spp.
256 DELURB2 lineage and *Plasmodium* spp. DELURB5 and PADOM01 lineages tended to
257 infect closely related bird species (Table 4).

258

Table 4: Phylogenetic dispersion of parasite lineages infecting more than one host species. *N* = number of bird species infected. Bolded values represent parasite lineages that were detected in close phylogenetic bird species.

	N	MPD_{obs}	Mean ± SD	*Z*	*P*
SGS1 *P. relictum*	9	79.976	81.829 ± 2.609	-0.710	0.222
PADOM05 *H. passeris*	**2**	**5.546**	**82.695±17.199**	**-4.485**	**0.002**
CCF2 *Haemoproteus* sp.	2	91.536	80.881±18.287	0.582	0.853
DELURB2 *Haemoproteus* sp.	**2**	**33.574**	**82.087±17.088**	**-2.838**	**0.038**
DELURB5 *Plasmodium* sp.	**2**	**33.574**	**82.111±17.851**	**-2.713**	**0.047**
GRW9 *Plasmodium* sp.	2	33.574	81.479±18.615	-2.573	0.054
HIPOL1 *Haemoproteus* sp.	2	86.983	81.817±17.714	0.291	0.304
PADOM1 *Plasmodium* sp.	**2**	**5.546**	**81.385±18.107**	**-4.188**	**0.003**

DISCUSSION

In this study of haemosporidian parasites infecting a community of wild birds breeding/migrating in the same area, we found that detected prevalence is mostly determined by the interaction between host and parasite identity. We also found that variation in prevalence might be affected by breeding season and parasite lineage. Additionally, we showed that *P. relictum* SGS1 is present in most of the bird species but with variable prevalence, while other parasites are more specialist parasites infecting fewer and close related host species as *H. passeris* PADOM05. Next, we discuss these main results in detail.

Virulence of *Plasmodium* and *Haemoproteus* spp. might be highly variable between species and lineages. Some malaria parasites might affect host fitness [55,56] and others might even influence survival [57–59]. Conversely, host bird species and bird individual might also differ in the prevalence displayed due to the tolerance or

276 susceptibility of each organism [11]. Thus, the interaction between parasite lineage and
277 host bird species ultimately determines infection severity and therefore prevalence. Our
278 results support this idea where variation in prevalence was mainly affected by the
279 interaction between parasite lineage and bird host species. In this sense, it has been
280 suggested that avian malaria parasites (*Plasmodium ashfordi*) express a particular host-
281 specific expression pattern depending on the infected bird [11]. This study highlighted
282 the role that the interaction between host immune defence (susceptibility or resistance to
283 parasites) and parasite strategy (virulence) may play in the prevalence displayed. The
284 mechanisms behind this host-parasite interaction are still limited where only some target
285 genes related, for instance, with red blood cell invasion have been deeply analysed
286 [60,61].

287 With regard to the previous results, prevalence was also affected by parasite
288 lineage. These results emphasize the importance not only of the host-parasite interaction
289 per se but also the parasite exploitation strategy that might vary depending on the bird
290 identity [11]. In this sense, it has been shown that avian malaria parasites might develop
291 a wide range of different strategies in order to avoid host defences. For example,
292 *Plasmodium* spp. can display some evasive mechanisms evading host defences by
293 clonal antigenic switches [62] or even avoid consequences of recognition by the
294 immune system [63]. Additionally, it has been shown that, during mix infections, these
295 parasite might compete with one another for access to the available hosts, thus, some
296 lineages show more prevalence in certain bird species simply because an specific
297 lineage may eliminate other parasite [23]. Hence, parasite lineage might play an
298 essential role determining the prevalence displayed in a host rather the host bird species
299 itself.

Our results also showed that breeding season affected prevalence displayed in the community. Previous studies have shown that, during breeding season prevalence of these parasite significantly increase [64,65] as a consequence of vector availability. The increase of vectors in the community may expand blood parasites among individuals and, therefore, increase prevalence [36]. Our results agree with this idea pointing out a clear connexion between breeding season, vector availability and prevalence of blood parasites.

It has been extensively discussed how specialist and generalist parasites may have evolved and how host species are selected to be infected [22,66,67]. Although specialist parasites are supposed to display higher prevalence and parasitemia on their avian hosts [21], generalist parasite can occasionally cause significant mortalities in native birds. For instance, in Hawaii birds *Plasmodium relictum* caused the extinction of native species of honeycreepers [68,69] despite being one of the most generalist parasite species. In this sense, it has been suggested that generalist parasites (*P. relictum* GRW4 and SGS1) have the ability to infect a broad range of host species but also display a high prevalence in some of them [70]. Our results agree with this hypothesis, where *P. relictum* SGS1 was detected in most of the bird species infected, and it displayed different mean prevalence depending on the bird species. Moreover, this haemosporidian lineage was detected in unrelated host species highlighting the parasite's ability to adapt to distantly related host species emphasizing the parasite's generalist strategy. In the case of *P. relictum* SGS1 the number of potential host species is 113 [15], where every host individual and species provide a different environment that need to be overcome by the plasticity of parasites [71]. Although the mechanism underlying this plasticity is still unknown, it has been recently shown that the reticulocyte binding proteins, a polymorphic gene family involved in host cell invasion

and attributed to host specificity [61], is significantly expanded in *P. relictum* [72]. This discovery could explain the ability of this parasite species to infect a wide range of avian species and its overwhelming capacity to infect a wide range of host species [72]. Nevertheless, further studies are needed in order to assess mechanisms allowing generalist parasites to be present in a large number of bird species.

Finally, our analysed stated that the number of infected bird species by the same parasite lineage do not determine whether this parasite may be more or less specialist. In this sense, *Haemoproteus* spp. CCF2, HIPOl1 lineages and *Plasmodium* spp. GRW9 were detected in distantly related bird species suggesting that, although each of these lineages was only detected in two bird species, they are not strict specialist parasites [32]. Additionally, *Haemoproteus* spp. PADOM05, DELURB2 lineages and *Plasmodium* spp. DELURB5, PADOM1 spp. lineages were detected in closely related bird species suggesting that these lineages may tent to be specialist parasites [32]. The prevalence displayed by these parasite lineages varied depending on the bird species infecting. Thus, in house sparrows (*Passer domesticus*) prevalence was higher for specialist parasites (*Haemoproteus* spp. PADOM05) than for generalist parasites. In contrast, Spanish house sparrows (*Passer hispaniolensis*) displayed prevalence was higher for generalist parasites (*P. relictum* SGS1). These results agree with previous studies suggesting that, in certain cases, specialist parasites might arise higher prevalence than generalist parasites [73] but, in other cases, generalist manage to reach high prevalence [70]. Our results can only confirm that depending on the parasite lineage and the host bird species prevalence might differ, suggesting that is the host-parasite interaction ultimately the factor that might affect prevalence in a wild community.

In conclusion, we have shown that, at the community level, the interaction between host and parasite identity might more strongly affect observed variation in prevalence than bird host species or parasite lineage alone. These results highlight the importance of studies focusing on the community level and analysing how different parasite lineages might interact with a variety of host species. The present study also highlights that even a parasite (such *as P. relictum* SGS1 and *H. passeris*. PADOM05) that are considered as generalist or a specialist can display both high and low prevalence per host species. Taken as a whole, these results suggest that prevalence mainly results from the interaction between host immune defences and parasite exploitation strategy where parasite lineage might play an important role following an approach depending on the bird species.

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368 **REFERENCES**

369

370 1. Hudson PJ, Rizzoli a. P, Grenfell BT, Heesterbeek J a. P, Dobson a. P. Ecology
371 of wildlife diseases. Ecol Wildl Dis. 2002;1981:1–5.
372 2. Hudson PJ, Dobson AP, Lafferty KD. Is a healthy ecosystem one that is rich in
373 parasites? Vol. 21, Trends in Ecology and Evolution. 2006. p. 381–5.
374 3. Schmid-Hempel P. Evolutionary parasitology: The integrated study of
375 infections, immunology, ecology and genetics. Vol. 27, Trends in parasitology. 2011.
376 233-4 p.
377 4. Bobby Fokidis H, Greiner EC, Deviche P. Interspecific variation in avian blood
378 parasites and haematology associated with urbanization in a desert habitat. J Avian Biol.
379 2008;39(3):300–10.
380 5. Prado-Irwin SR, Bird AK, Zink AG, Vredenburg VT. Intraspecific Variation in
381 the Skin-Associated Microbiome of a Terrestrial Salamander. Microb Ecol.
382 2017;74(3):745–56.
383 6. Olsson-Pons S, Clark NJ, Ishtiaq F, Clegg SM. Differences in host species
384 relationships and biogeographic influences produce contrasting patterns of prevalence,
385 community composition and genetic structure in two genera of avian malaria parasites
386 in southern Melanesia. J Anim Ecol. 2015;84(4):985–98.
387 7. Valkiūnas G. Avian malaria parasites and other Haemosporidia. Boca Raton.
388 2005.
389 8. Sehgal RNM. Manifold habitat effects on the prevalence and diversity of avian
390 blood parasites. Vol. 4, International Journal for Parasitology: Parasites and Wildlife.
391 2015. p. 421–30.

392 9. Matthews AE, Ellis VA, Hanson AA, Roberts JR, Ricklefs RE, Collins MD.
393 Avian haemosporidian prevalence and its relationship to host life histories in eastern
394 Tennessee. J Ornithol. 2016;157(2):533–48.
395 10. Wilkinson LC, Handel CM, Van Hemert C, Loiseau C, Sehgal RNM. Avian
396 malaria in a boreal resident species: long-term temporal variability, and increased
397 prevalence in birds with avian keratin disorder. Int J Parasitol. 2016 Apr 1 [cited 2018
398 Mar 19];46(4):281–90.
399 11. Videvall E, Cornwallis CK, Ahrén D, Palinauskas V, Valkiunas G, Hellgren O.
400 The transcriptome of the avian malaria parasite Plasmodium ashfordi displays host-
401 specific gene expression. Molecular Ecology. 2017;
402 12. Hellgren O, Križanauskienė A, Hasselquist D, Bensch S. LOW
403 HAEMOSPORIDIAN DIVERSITY AND ONE KEY-HOST SPECIES IN A BIRD
404 MALARIA COMMUNITY ON A MID-ATLANTIC ISLAND (SÃO MIGUEL,
405 AZORES). J Wildl Dis. 2011;47(4):849–59.
406 13. Waldenström J, Bensch S, Kiboi S, Hasselquist D, Ottosson U. Cross-species
407 infection of blood parasites between resident and migratory songbirds in Africa. Mol
408 Ecol. 2002;11(8):1545–54.
409 14. Atkinson CT, Saili KS, Utzurrum RB, Jarvi SI. Experimental evidence for
410 evolved tolerance to avian malaria in a wild population of low elevation Hawai'i
411 'Amakihi (Hemignathus virens). Ecohealth. 2013;10(4):366–75.
412 15. Bensch S, Hellgren O, Pérez-Tris J. MalAvi: a public database of malaria
413 parasites and related haemosporidians in avian hosts based on mitochondrial
414 cytochrome b lineages. Mol Ecol Resour. 2009;9:1353–8.
415 16. Palinauskas V, Žiegytė R, Iezhova TA, Ilgūnas M, Bernotienė R, Valkiūnas G.
416 Description, molecular characterisation, diagnostics and life cycle of Plasmodium

417 elongatum (lineage pERIRUB01), the virulent avian malaria parasite. Int J Parasitol.
418 2016;46(11):697–707.
419 17. Reece SE, Ali E, Schneider P, Babiker HA. Stress, drugs and the evolution of
420 reproductive restraint in malaria parasites. Proc R Soc B Biol Sci.
421 2010;277(1697):3123–9.
422 18. Pollitt LC, Mideo N, Drew DR, Schneider P, Colegrave N, Reece SE.
423 Competition and the Evolution of Reproductive Restraint in Malaria Parasites. Am Nat.
424 2011;177(3):358–67.
425 19. Cornet S, Nicot A, Rivero A, Gandon S. Evolution of Plastic Transmission
426 Strategies in Avian Malaria. PLoS Pathog. 2014;10(9).
427 20. Combes C. Parasitism: The ecology and evolution of intimate interactions. Vol.
428 14, Parasitism The ecology and evolution of intimate interactions. 2001. 728 p.
429 21. Keesing F, Holt RD, Ostfeld RS. Effects of species diversity on disease risk.
430 Vol. 9, Ecology Letters. 2006. p. 485–98.
431 22. Fallon SM, Bermingham E, Ricklefs RE. Host specialization and geographic
432 localization of avian malaria parasites: a regional analysis in the Lesser Antilles. Am
433 Nat. 2005;165(4):466–80.
434 23. Fallon SM, Ricklefs RE, Latta SC, Bermingham E. Temporal stability of insular
435 avian malarial parasite communities. Proc R Soc B Biol Sci . 2004;271(1538):493–500.
436 24. Ricklefs RE, Swanson BL, Fallon SM, Martínez-Abraín A, Scheuerlein A, Gray
437 J, et al. Community relationships of avian malaria parasites in southern Missouri. Ecol
438 Monogr. 2005;75(4):543–59.
439 25. Moens MAJ, Pérez-Tris J. Discovering potential sources of emerging pathogens:
440 South America is a reservoir of generalist avian blood parasites. Int J Parasitol.
441 2016;46(1):41–9.

442 26. Fecchio A, Bell JA, Collins MD, Farias IP, Trisos CH, Tobias JA, et al.
443 Diversification by host switching and dispersal shaped the diversity and distribution of
444 avian malaria parasites in Amazonia. Oikos. 2018;
445 27. Ricklefs RE, Outlaw DC, Svensson-Coelho M, Medeiros MCI, Ellis VA, Latta
446 S. Species formation by host shifting in avian malaria parasites. Proc Natl Acad Sci.
447 2014;111(41):14816–21.
448 28. Alan F, Jeffrey Andrew B, Michael David C, Izeni Pires F, Christopher Harry T,
449 Joseph Andrew T, et al. Diversification by host switching and dispersal shaped the
450 diversity and distribution of avian malaria parasites in Amazonia. Oikos. 2018;00:1–10.
451 29. Medeiros MCI, Hamer GL, Ricklefs RE. Host compatibility rather than vector-
452 host-encounter rate determines the host range of avian Plasmodium parasites. Proc R
453 Soc B Biol Sci . 2013;280(1760):20122947–20122947.
454 30. Clark NJ, Clegg SM. Integrating phylogenetic and ecological distances reveals
455 new insights into parasite host specificity. Mol Ecol. 2017;26(11):3074–86.
456 31. Møller AP, Merino S, Brown CR, Robertson RJ. Immune Defense and Host
457 Sociality: A Comparative Study of Swallows and Martins. Am Nat . 2001;158(2):136–
458 45.
459 32. Svensson-Coelho M, Loiselle BA, Blake JG, Ricklefs RE. Resource
460 predictability and specialization in avian malaria parasites. Mol Ecol.
461 2016;25(17):4377–91.
462 33. Moens MAJ, Valkiūnas G, Paca A, Bonaccorso E, Aguirre N, Pérez-Tris J.
463 Parasite specialization in a unique habitat: hummingbirds as reservoirs of generalist
464 blood parasites of Andean birds. J Anim Ecol. 2016;85(5):1234–45.
465 34. Pulgarín-R PC, Cadena CD, Gómez JP, Robinson S, Ricklefs RE. Host species ,
466 and not environment , predicts variation in blood parasite prevalence , distribution , and

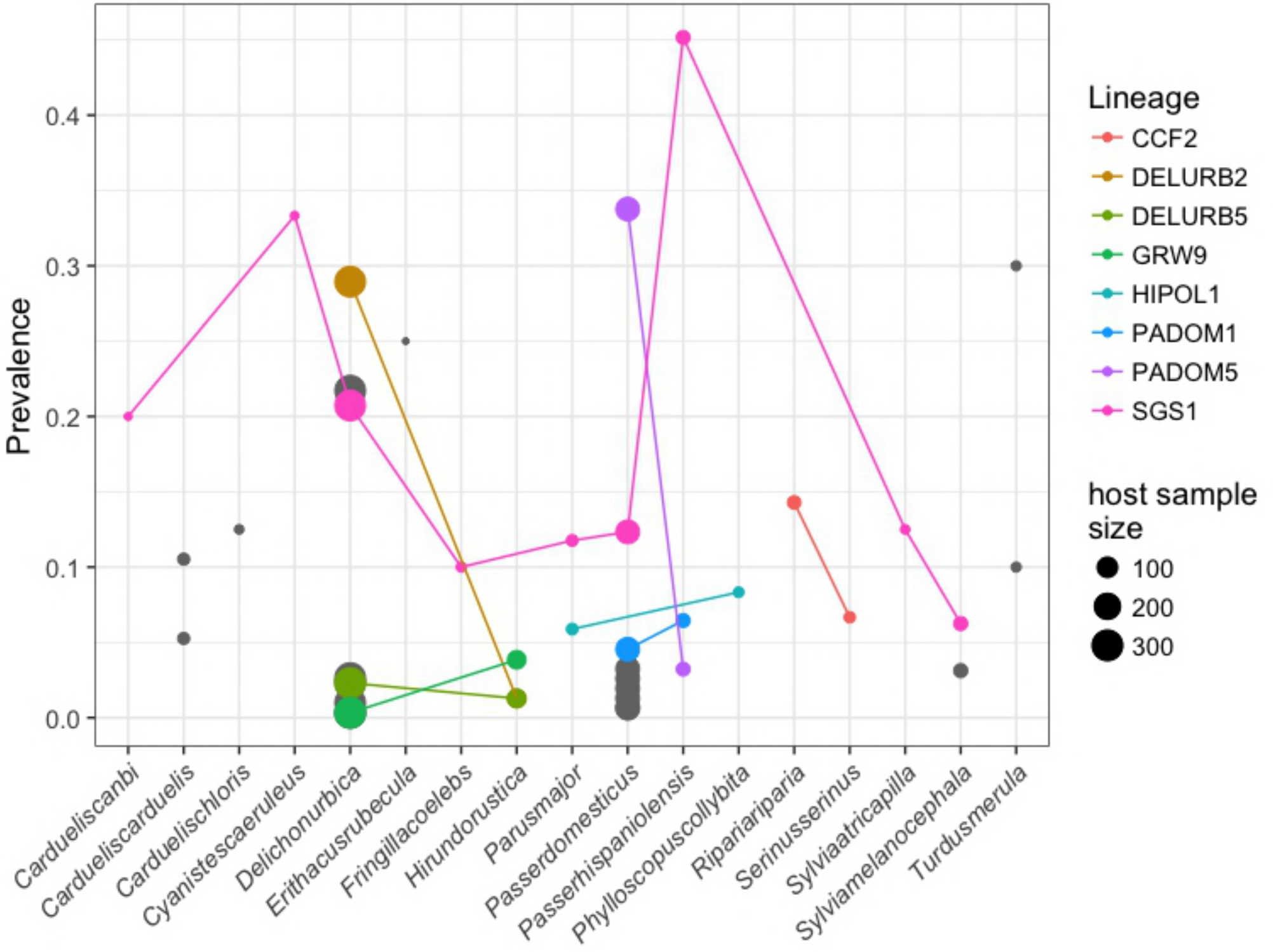
467 diversity along a humidity gradient in northern South America. Ecol Evol.
468 2018;(December 2017):1–15.
469 35. Garamszegi LZ. Climate change increases the risk of malaria in birds. Glob
470 Chang Biol. 2010 Nov 10 [cited 2011 Mar 16];no-no.
471 36. Ferraguti M, Martínez-de la Puente J, Muñoz J, Roiz D, Ruiz S, Soriguer R, et
472 al. Avian Plasmodium in Culex and Ochlerotatus Mosquitoes from Southern Spain:
473 Effects of Season and Host-Feeding Source on Parasite Dynamics. PLoS One.
474 2013;8(6).
475 37. Van Rooyen J, Jenkins T, Lahlah N, Christe P. North-african house martins
476 endure greater haemosporidian infection than their european counterparts. Journal of
477 Avian Biology. 2014;
478 38. Schmid-Hempel P. Parasites and Their Social Hosts. Vol. 33, Trends in
479 Parasitology. 2017. p. 453–62.
480 39. Sambrook J, Fritsch EF, Maniatis T. Molecular Cloning: A Laboratory Manual.
481 Cold Sprin. Vol. 3, Cold Spring Harbor Laboratory. 2002. 2344 p.
482 40. Waldenström J, Bensch S, Hasselquist D, Ostman O. A new nested polymerase
483 chain reaction method very efficient in detecting Plasmodium and Haemoproteus
484 infections from avian blood. J Parasitol. 2004;90:191–4.
485 41. Bensch S, Stjernman M, Hasselquist D, Ostman O, Hansson B, Westerdahl H, et
486 al. Host specificity in avian blood parasites: a study of Plasmodium and Haemoproteus
487 mitochondrial DNA amplified from birds. Proc R Soc B Biol Sci. 2000;267:1583–9.
488 42. Hall T. BioEdit: a user-friendly biological sequence alignment editor and
489 analysis program for Windows 95/98/NT. Nucleic Acids Symp Ser. 1999;41:95–8.

490 43. Bensch S, Pérez-Tris J, Waldenström J, Hellgren O. Linkage between nuclear
491 and mitochondrial DNA sequences in avian malaria parasites: multiple cases of cryptic
492 speciation? Evolution. 2004;58(7):1617–21.
493 44. Jetz W, Thomas GH, Joy JB, Hartmann K, Mooers AO. The global diversity of
494 birds in space and time. Nature. 2012;491(7424):444–8.
495 45. Kearse M, Moir R, Wilson A, Stones-Havas S, Cheung M, Sturrock S, et al.
496 Geneious Basic: An integrated and extendable desktop software platform for the
497 organization and analysis of sequence data. Bioinformatics. 2012;28(12):1647–9.
498 46. Huelsenbeck JP, Ronquist F. MRBAYES: Bayesian inference of phylogenetic
499 trees. Bioinformatics. 2001;17(8):754–5.
500 47. Ronquist F, Huelsenbeck JP. MrBayes 3: Bayesian phylogenetic inference under
501 mixed models. Bioinformatics. 2003;19(12):1572–4.
502 48. Posada D. jModelTest: Phylogenetic Model Averaging. Mol Biol Evol.
503 2008;25(7):1253–6.
504 49. Rambaut A, Drummond A. Tracer - MCMC Trace Analysis Tool, v1.4. Tracer –
505 MCMC Trac. 2007.
506 50. Hadfield JD. MCMC methods for multi-response generalized linear mixed
507 models: the MCMCglmm R package. J Stat Softw. 2010;33(2):1–22.
508 51. Videvall E, Cornwallis CK, Palinauskas V, Valkiunas G, Hellgren O. The avian
509 transcriptome response to malaria infection. Mol Biol Evol. 2015;32(5):1255–67.
510 52. Wilkinson L. ggplot2: Elegant Graphics for Data Analysis by WICKHAM, H.
511 Biometrics . 2011;67(2):678–9.
512 53. R Development Core Team. R: A language and environment for statistical
513 computing. R Foundation Statistical Computing. Vienna, Austria; 2011.

514 54. Kembel SW, Cowan PD, Helmus MR, Cornwell WK, Morlon H, Ackerly DD, et
515 al. Picante: R tools for integrating phylogenies and ecology. Bioinformatics.
516 2010;26(11):1463–4.
517 55. Marzal A, de Lope F, Navarro C, Møller AP. Malarial parasites decrease
518 reproductive success: an experimental study in a passerine bird. Oecologia. 2005
519 Feb;142(4):541–5.
520 56. Lachish S, Knowles SCL, Alves R, Wood MJ, Sheldon BC. Fitness effects of
521 endemic malaria infections in a wild bird population: the importance of ecological
522 structure. J Anim Ecol. 2011;80:1196–206.
523 57. Puente JM -d. l., Merino S, Tomas G, Moreno J, Morales J, Lobato E, et al. The
524 blood parasite Haemoproteus reduces survival in a wild bird: a medication experiment.
525 Biol Lett. 2010;6(5):663–5.
526 58. Asghar M, Hasselquist D, Hansson B, Zehtindjiev P, Westerdahl H, Bensch S.
527 Hidden costs of infection: Chronic malaria accelerates telomere degradation and
528 senescence in wild birds. Science. 2015;347(6220):436–8.
529 59. Marzal A, Balbontín J, Reviriego M, García-Longoria L, Relinque C, Hermosell
530 IG, et al. A longitudinal study of age-related changes in Haemoproteus infection in a
531 passerine bird. Oikos. 2016;125(8).
532 60. Beeson JG, Drew DR, Boyle MJ, Feng G, Fowkes FJI, Richards JS. Merozoite
533 surface proteins in red blood cell invasion, immunity and vaccines against malaria. Vol.
534 40, FEMS Microbiology Reviews. 2016. p. 343–72.
535 61. Otto TD, Rayner JC, Bï¿½hme U, Pain A, Spottiswoode N, Sanders M, et al.
536 Genome sequencing of chimpanzee malaria parasites reveals possible pathways of
537 adaptation to human hosts. Nat Commun. 2014;5.

538 62. Guizetti J, Scherf A. Silence, activate, poise and switch! Mechanisms of
539 antigenic variation in Plasmodium falciparum. Vol. 15, Cellular Microbiology. 2013. p.
540 718–26.
541 63. Hisaeda H, Maekawa Y, Iwakawa D, Okada H, Himeno K, Kishihara K, et al.
542 Escape of malaria parasites from host immunity requires CD4+ CD25+ regulatory T
543 cells. Nat Med. 2004;10(1):29–30.
544 64. Deviche P, Greiner EC, Manteca X. Interspecific Variability of Prevalence in
545 Blood Parasites of Adult Passerine Birds During the Breeding Season in Alaska. J Wildl
546 Dis. 2001;37(1):28–35.
547 65. Szöllősi E, Garamszegi LZ, Hegyi G, Laczi M, Rosivall B, Török J.
548 Haemoproteus infection status of collared flycatcher males changes within a breeding
549 season. Parasitol Res. 2016;115(12):4663–72.
550 66. Loiseau C, Harrigan RJ, Robert A, Bowie RCK, Thomassen HA, Smith TB, et
551 al. Host and habitat specialization of avian malaria in Africa. Mol Ecol.
552 2012;21(2):431–41.
553 67. Clark NJ, Olsson-Pons S, Ishtiaq F, Clegg SM. Specialist enemies, generalist
554 weapons and the potential spread of exotic pathogens: Malaria parasites in a highly
555 invasive bird. Int J Parasitol. 2015;45(14):891–9.
556 68. Atkinson CT, Lapointe DA. Introduced avian diseases, climate change, and the
557 future of Hawaiian honeycreepers. J Avian Med Surg. 2009;23(1):53–63.
558 69. Van Riper III C, Van Riper SG, Goff ML, Laird M. The Epizootiology and
559 Ecological Significance of Malaria in Hawaiian Land Birds. Ecol Monogr.
560 1986;56:327–44.

561 70. Hellgren O, Pérez-Tris J, Bensch S. A jack-of-all-trades and still a master of
562 some: Prevalence and host range in avian malaria and related blood parasites. Ecology.
563 2009;90(10):2840–9.
564 71. Richards CL, Bossdorf O, Muth NZ, Gurevitch J, Pigliucci M. Jack of all trades,
565 master of some? On the role of phenotypic plasticity in plant invasions. Vol. 9, Ecology
566 Letters. 2006. p. 981–93.
567 72. Böhme U, Otto TD, Cotton JA, Steinbiss S, Sanders M, Oyola SO, et al.
568 Complete avian malaria parasite genomes reveal features associated with lineage-
569 specific evolution in birds and mammals. Genome Res. 2018;28:1.14.
570 73. Medeiros MCI, Ellis V a., Ricklefs RE. Specialized avian Haemosporida trade
571 reduced host breadth for increased prevalence. J Evol Biol. 2014;27(11):2520–8.
572


Prevalence
0.0
0.1
0.2
0.3
0.4
Carduelis canbi
Carduelis carduelis
Carduelis chloris
Cyanistes caeruleus
Delichon urbica
Erithacus rubecula
Fringilla coelebs
Hirundo rustica
Parus major
Passer domesticus
Passer hispaniolensis
Phylloscopus collybita
Riparia riparia
Serinus serinus
Sylvia atricapilla
Sylvia melanocephala
Turdus merula
Lineage
CCF2
DELURB2
DELURB5
GRW9
HIPOL1
PADOM1
PADOM5
SGS1
host sample size
100
200
300