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Testing epidemiological functional groups as predictors of avian haemosporidia patterns in southern Africa

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Abstract. Understanding the dynamics of multihost parasites and the roles of different host species in parasite epidemiology requires consideration of the whole animal community. Host communities may be composed of hundreds of interacting species, making it necessary to simplify the problem. One approach to summarizing the host community in a way that is relevant to the epidemiology of the parasite is to group host species into epidemiological functional groups (EpiFGs). We used EpiFGs to test our understanding of avian malaria (*Plasmodium* and *Haemoproteus*) dynamics in four communities of wetland-associated birds in southern Africa. Bird counts and captures were undertaken every 2–4 months over 2 yr and malaria was diagnosed by nested PCR. One hundred and seventy-six bird species were allocated to a set of EpiFGs according to their assumed roles in introducing and maintaining the parasite in the system. Roles were quantified as relative risks from avian foraging, roosting, and movement ecology and assumed interaction with vector species. We compared our estimated a priori risks to empirical data from 3414 captured birds from four sites and 3485 half-hour point counts. After accounting for relative avian abundance, our risk estimates significantly correlated with the observed prevalence of *Haemoproteus* but not *Plasmodium*. Although avian roosting height (for both malarial genera) and movement ecology (for *Plasmodium*) separately influenced prevalence, host behavior alone was not sufficient to predict *Plasmodium* patterns in our communities. Host taxonomy and relative abundance were also important for this parasite. Although using EpiFGs enabled us to predict the infection patterns of only one genus of heamosporidia, our approach holds promise for examining the influence of host community composition on the transmission of vector-borne parasites and identifying gaps in our understanding of host–parasite interactions.

Key words: avian community; avian malaria; *Haemoproteus*; multihost parasites; *Plasmodium*; relative risk; southern Africa.

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Introduction

Host–parasite interactions are often considered as "one host–one parasite" systems. However, most parasites are capable of infecting multiple hosts (Woolhouse et al. 2001), and most hosts support multiple parasites (Cox 2001), creating considerable potential for complex interactions. Understanding parasite prevalence, impact, and evolution, and managing infectious diseases,

requires the adoption of both an evolutionary and a whole-ecosystem view of host–parasite interactions (Wilcox and Colwell 2005, Ostfeld et al. 2008, Johnson et al. 2015). The importance of a holistic approach is particularly apparent where the anthropogenic modification of natural habitats has led to loss of biodiversity and increased contacts between wild and domestic species and humans (Cleaveland et al. 2001). As the composition of host communities is altered (Child et al. 2009), so is that of parasite communities (Kwa 2008), creating the potential for rapid changes in the dynamics of infectious diseases (Lambin et al. 2010).

The dynamics of multihost parasites in host communities are complex and poorly understood (Woolhouse et al. 2001). The influence of species richness and diversity on parasite abundance remains highly debated (Begon 2008, Keesing et al. 2010, Salkeld et al. 2013, Wood and Lafferty 2013). Different studies have shown contrasting results and several possible mechanisms by which host community composition may influence that of parasites (Randolph and Dobson 2012, Wood and Lafferty 2013). One emerging finding is that the identities and abundances of host species may be more important than their biodiversity per se (LoGiudice et al. 2008, Roche et al. 2013, Salkeld et al. 2013), suggesting a need to better understand the impact of host community structure and composition on the dynamics of multihost parasites.

Host species can have multiple roles in the epidemiology of a parasite. Parasite maintenance is usually favored by particular host species. Their identification has been the focus of many theoretical studies but remains a challenge empirically (Viana et al. 2014), in part because interpretation of the epidemiological role of a species cannot be done rigorously without considering the potential roles of the other species in the same community. In some multihost systems, so called "bridge hosts" can link maintenance and susceptible hosts (Caron et al. 2015), (re)introducing new parasite strains to host populations or spreading them to new ecosystems, where naïve species may be highly impacted (e.g., Atkinson et al. 2001). Dead-end hosts (infected but not transmitting) may reduce the overall prevalence of the parasite in the community, as may competitors and predators of infected hosts, unless the parasite uses interspecific interactions as transmission modes.

Trying to include all host species in an analysis means dealing with hundreds of potential interactions, their number increasing exponentially with the number of species. Ways of simplifying the problem are therefore needed. Community ecologists have developed a range of approaches to deconstructing the complexity of food webs using foraging guilds or trophic levels (May 2006). Disease ecologists have recently started using network approaches where hosts are represented by nodes linked by edges summarizing qualitatively or quantitatively the level of shared pathogen (Caron et al. 2012*a*). From an epidemiological perspective, one can also summarize host communities using species epidemiological functions (EFs) (e.g., maintenance, introduction) rather than their foraging guilds (Caron et al. 2010). Epidemiological functional groups (EpiFGs) allow ecologists to summarize the host community in a way that makes sense for the epidemiology of parasites without oversimplifying it (Caron et al. 2012*b*).

An analysis of the ecology of avian influenza based on EpiFGs revealed inconsistencies between current knowledge of the disease and empirical data (Caron et al. 2012*b*). We extended this approach to use EpiFGs to test our understanding of avian malaria dynamics in communities of wetland-associated birds in southern Africa, and to identify future research priorities. Avian malaria has been largely overlooked in community-level analysis, both globally and in southern Africa, although it infects a wide range of bird species and Sub-Saharan Africa is one of its main transmission areas (Valkiunas 2005).

Unlike avian influenza, avian malaria is a vector-borne parasite. Applying the EpiFG approach to this system thus requires considering both bird and vector ecology and renders our analysis more complex. For ease of writing we use "avian malaria" to refer to two genera of avian haematozoa, *Plasmodium* and *Haemoproteus*, noting that many researchers reserve the term "malaria" solely for *Plasmodium* infections. Both *Plasmodium* and *Haemoproteus* are intracellular protozoan blood parasites that infect a wide range of birds worldwide. Their complex life cycles include asexual stages of reproduction in a bird host and sexual stages within a vector

(Valkiunas 2005). *Plasmodium* spp. are transmitted between birds by blood-sucking mosquitoes, mainly from the Culicine family (Valkiunas 2005). *Coquilletidia* spp. have recently been identified as important vectors for avian malaria in Africa (Njabo et al. 2009). *Haemoproteus* spp. are transmitted by *Culicoides* biting midges and hippoboscid flies (Valkiunas 2005).

We used EpiFGs to test the hypothesis that epidemiologically relevant ecological traits of the host species (e.g., roosting and foraging behavior) can predict their likelihood of infection with avian malaria. We would expect that in a system in which parasites are relatively generalist, host ecology drives host–parasite contacts and that resulting trends in infection levels will emerge at the community level. The competing hypothesis, although not mutually exclusive, suggests that the evolution of host–parasite dynamics in this system is driven by host physiology, taxonomy, co-evolutionary history, and/or compatibility at the host–parasite interface, independent of host ecology. Our results have general implications for the use of EpiFGs in epidemiology as well as for our understanding of avian malaria.

Materials and Methods

Field sites

Bird counts and sampling were conducted at four perennial wetlands in southern Africa: (1) Barberspan Nature Reserve (BAR), a RAMSAR wetland in the North West Province in South Africa; (2) Strandfontein wastewater treatment works (STR), next to the city of Cape Town in South Africa, where birds use an old network of ponds; (3) the Manyame and Chivero Dams (ZIM) in Zimbabwe, man-made impoundments linked by the Manyame River and built to supply the city of Harare with water and (4) Lake Ngami (NGA), a "dead-end" lake with no outflow located at the southern end of the Okavango system in Botswana. All sites except STR receive rainfall during summer (November–March). STR is in a winter-rainfall region, with peak rainfall in July. More information is available as supplementary material in (Cumming et al. 2011).

Birds censuses and sampling

Standardized point counts were carried out between February 2007 and April 2009 every 2 (BAR, STR, ZIM) or 4 months (NGA). Each focal count consisted of a 10-min habituation period followed by a 30-min point count of all birds in a semicircle of 150 m radius (facing the waterbody). Counts were undertaken at 12–15 points per site and repeated four times each over 5 d during each session, totaling 3485 half-hour counts.

Immediately after each counting session, a week of intensive capture and sampling was carried out. Wild birds were caught using walk-in traps, mist nests and occasionally whoosh- or cannon-nets placed near the water's edge. Blood was collected from the brachial vein and preserved in vials containing an SDS lysis buffer. Birds were ringed to identify potential recaptures and released after sampling. As the protocol was initially designed to study the role of Anseriforms and Charadriiforms in Avian Influenza epidemiology, methods were chosen to maximize waterbird captures, although all "bycatch" species were sampled.

From the 384 bird species counted over the four sites (151–261 species/site), 176 (from 20 orders and 65 families) were included in the analysis. We excluded rare species (counted in <2 sessions) and species for which ecological knowledge was insufficient. Included species were known to be competent hosts of avian haemosporidia, i.e., tested positive in a previous study or in this one (references in Appendix S1). From the 176 species considered, 86 were tested for avian malaria.

Avian malaria diagnosis

Blood samples from 3427 individuals were screened for avian malaria using nested PCR, as detailed in (Cumming et al. 2013). DNA was extracted using the DNeasy blood and tissue kit (Quiagen). A nested PCR was used to target a 478 bp fragment of the mitochondrial cytochrome b gene from the genera *Haemoproteus* and *Plasmodium*, following the protocol of (Waldenström et al. 2002). Automated sequencing was performed at the DNA sequencing facility on Science Hill at Yale University on an ABI 3730. Two trials were conducted for each sample. Sequences were aligned and edited using Sequencher (Gene Codes Corporation, Ann Arbor, Michigan, USA).

Table 1. Epidemiological functional groups (bird species can belong to more than one group).

Epidemiological function	Epidemiological functional group	rr.P†	rr.H†	N_{spC}‡	N_{spT}‡	n_T§	Prev.P (%)¶	Prev.H (%)¶
Introducers	Residents (*res*)	H1:1; H2: 3	H1:1; H2: 3	30	11	44	25.00	7.50
Introducing strains into the ecosystem from other ecosystems	Local and middle-range spreaders (nomads and Afrotropical migrants) (*afro*)	H1:2; H2: 2	H1:2; H2: 2	125	67	3289	3.76	3.15
	Long-range spreaders (palaearctic migrants) (*pal*)	H1: 3; H2: 1	H1: 3; H2: 1	21	8	81	0.00	0.00
Maintainers	Aerial foragers (*aer*)	1	1	7	0	0	na	na
Capable of maintaining the parasite in the community. Assumed to be competent and highly exposed species.	Aquatic foragers (*aqua*)	2	2	49	35	2149	4.44	0.51
	Terrestrial foragers (*terr*)	3	3	120	51	1265	3.04	7.59
	Ground roosters (≤1 m above ground) (*gr*)	3	1	67	45	2505	4.18	1.12
	Mid-story roosters (1–7 m above ground) (*mid*)	2	2	72	29	753	3.49	6.57
	Canopy roosters (≥7 m above ground) (*can*)	1	3	37	12	156	1.94	18.71

† Relative risks of infection by *Plasmodium* (rr.P) and *Haemoproteus* (rr.H). For the introducers group, two hypotheses were tested: (1) H1: haemosporidia dynamics are mostly driven by exogeneous strains and (2) H2: haemosporidia dynamics are mostly driven by local strains.

‡ Number of counted (N_{spC}) and tested (N_{spT}) species

§ Number of tested individuals.

¶ Observed *Plasmodium* (Prev.P) and *Haemoproteus* (Prev.H) prevalence.

Data analysis

Epidemiological functional groups for Plasmodium *and* Haemoproteus.—We allocated the 176 bird species to nine EpiFGs according to two EFs: introduction and maintenance of avian malaria (several EpiFGs exist for each EF; Table 1). Within each EF, we allocated a priori relative risks for avian malaria to each EpiFG (from 1 [a small risk] to 3 [a high risk]), based on current mainstream understanding of avian malaria and vector ecology, as detailed below. Given their differences in life cycle and vectors, we created different EpiFGs for *Plasmodium* and *Haemoproteus*, respectively.

First, migratory birds have been shown to carry many parasites, including haemosporidia (Valkiunas 2005, Altizer et al. 2011). Highly mobile competent birds could introduce new strains of *Plasmodium* or *Haemoproteus* from different ecosystems across regions and continents. We allocated birds into three EpiFGs according to their movement ecology as defined in the seventh edition of the Roberts' Birds of Southern Africa (Hockey et al. 2005). As little is known about the dynamics of haemosporidia in southern Africa, two extreme hypotheses were tested: (1) H1: haemosporidia dynamics are driven by exogeneous strains and (2) H2: haemosporidia dynamics are driven by local strains. Under H1, long-range spreaders or Palaearctic migrants, migrating from Eurasia, are the most likely to be exposed to the parasites and to introduce exogenous strains and were therefore allocated the maximum relative risk. Middle-range spreaders or Afrotropical migrants, local spreaders, and nomadic species were all allocated an intermediate relative risk. We grouped these latter species into a single group because the movement ecology of many species in southern Africa remains unclear and because several species exhibit regional spatial or temporal variation in their movement strategies (Cumming et al. 2012). Non-spreaders or resident species were allocated the minimum relative risk. As avian mobility is a host trait rather than a parasite trait, the same relative risks were attributed to hosts for both genera of malaria. Under H2, resident species are more exposed to local strains and were allocated the highest relative risk, while afrotropical migrants and nomadic species were allocated an intermediate risk and Palearctic migrants the minimum risk.

Second, we assumed that competent birds highly exposed to vectors by their behavior were potential maintainers of the disease. They were

allocated according to their foraging and roosting ecology based on (Hockey et al. 2005). We used known variations in abundance of adult vectors according to substratum and height and their temporal activity patterns to estimate the relative exposure risks of bird species. We did not attempt to identify the maintenance community of avian malaria but rather propose a way to group the species that are the most likely to participate in the maintenance of the parasite according to their behavioral characteristics. Birds were allocated to one of three EpiFGs depending on the substratum on which they forage (Hockey et al. 2005). First, most waterbirds forage during the day, when most mosquitoes and biting midges are less active and seek shelter in low vegetation, cracks and holes in the ground or bushes up to about a meter high (Silver 2007). Birds foraging on a terrestrial substratum or just off the ground were thus assumed to be the most likely to encounter adult vectors and were allocated the highest relative risk of infection for both *Plasmodium* and *Haemoproteus*. Such birds can also be targeted by vectors throughout their foraging time, unlike species that hunt in flight. Second, birds foraging on the water surface or on muddy edges were considered to be highly exposed to vectors, but less than ground foragers. Although no study has investigated the abundance of adult mosquitoes or biting midges on the water surface, we assumed that adult vectors would be less abundant on water than on land because of increased wind exposure and the lack of landing sites. Third, as birds with an aerial foraging strategy present difficult moving targets, they were expected to be the least exposed to *Plasmodium* and *Haemoproteus*.

Malaria vectors exhibit a strong vertical stratification when seeking for hosts at night (Mellor et al. 2000, Sinka et al. 2010), which should differentially expose birds, depending on their roosting height. As previous studies suggest differences in the vertical distribution of *Plasmodium* and *Haemoproteus* vectors, different relative risks were attributed depending on the malaria genus being considered. Host-seeking individuals of most mosquitoes are more abundant below 1 m above ground and their abundance decreases with height (Gillies and Wilkes 1976, Cerný et al. 2011). Some *Culex* and *Anopheles* species are however more abundant at medium heights (e.g., *Culex neavei Theo*, *Anopheles Giles*; Gillies and Wilkes 1976) and some *Culex* are found more at the canopy level (>7 m above ground) (e.g., *Culex pipiens, Culex tarsalis* Coquillette; Meyers 1959, Gillies and Wilkes 1976). Accordingly, we allocated hosts to three EpiFGs as a function of their roosting height (Hockey et al. 2005), with birds roosting on the ground being the most at risk to *Plasmodium*; birds roosting at the mid-story being intermediate; and birds roosting at the canopy level being the least at risk. Biting midges tend to be more abundant at canopy level and then decrease in abundance with decreasing height (Garvin and Greiner 2003, Cerný et al. 2011), although fewer studies have been conducted on those species. Accordingly, we considered birds roosting at canopy level as the most at risk to *Haemoproteus*; birds roosting at the mid-story level as intermediate; and birds roosting on the ground as the least at risk.

Body mass is sometimes considered as a risk factor for vector-borne diseases as larger birds may be easier targets for blood-feeding vectors (Atkinson and Van Riper 1991). However previous studies on avian haemosporidia showed contradictory results (Schrader et al. 2003, Schultz et al. 2010) and preliminary analyses showed that including the average mass of species did not change the results presented here. Hence, body mass was not considered in the creation of the maintainers group.

A bird species may have more than one function in the epidemiology of a parasite and can thus belong to more than one EpiFG. Its infection risk is the result of a combination of ecological characteristics that determine its exposure to the pathogen. The relative risk of each species i (rr_i) was calculated by multiplying its relative risks of introduction ($rr_{i,intro}$), maintenance knowing its foraging ecology ($rr_{i,maint|for}$), and maintenance knowing its roosting ecology ($rr_{i,maint|roo}$):

$$rri = rr_{i,intro} \times rr_{i,maint|for} \times rr_{i,maint|roo} \quad (1)$$

The host community can be summarized by grouping species having the same EpiFG combination and therefore the same total relative risk (Table 2; see Appendix S1: Table S1 for the relative risk of all species). Note that by multiplying the risks of the EpiFGs related to introduction and

Table 2. Epidemiological functional group combinations (combinations with ≥12 sampled individuals globally are in bold, those not tested are indicated by "na").

Epidemiological functional groups combination	Abbreviation	rr.P†		rr.H†		N_{spC}‡	N_{spT}‡	n_T§	Prev.P (%)¶	Prev.H (%)¶
		H1	H2	H1	H2					
Resident–Aquatic forager–Ground rooster	res-aqua-gr	6	18	2	6	1	0	0	na	na
Resident–Terrestrial forager–Ground rooster	**res-terr-gr**	**9**	**27**	**3**	**9**	**13**	**5**	**21**	**23.81**	**9.52**
Resident–Terrestrial forager–Canopy rooster	res-terr-can	3	9	9	27	14	0	0	na	na
Resident–Terrestrial forager–Mid-story rooster	**res-terr-mid**	**6**	**18**	**6**	**18**	**2**	**6**	**23**	**26.32**	**5.26**
Afrotropical migrant–Aquatic forager–Mid-story rooster	afro-aqua-mid	8	8	8	8	4	0	0	na	na
Afrotropical migrant–Aerial forager–Canopy rooster	afro-aer-can	2	2	6	6	1	0	0	na	na
Afrotropical migrant–Aquatic forager–Ground rooster	**afro-aqua-gr**	**12**	**12**	**4**	**4**	**24**	**22**	**2036**	**4.63**	**0.49**
Afrotropical migrant–Aquatic forager–Mid-story rooster	**afro-aqua-mid**	**8**	**8**	**8**	**8**	**9**	**2**	**14**	**7.14**	**0**
Afrotropical migrant–Aquatic forager–Canopy rooster	**afro-aqua-can**	**4**	**4**	**12**	**12**	**4**	**3**	**18**	**0**	**5.56**
Afrotropical migrant–Terrestrial forager–Ground rooster	**afro-terr-gr**	**18**	**18**	**6**	**6**	**18**	**10**	**367**	**1.39**	**4.43**
Afrotropical migrant–Terrestrial forager–Mid-story rooster	**afro-terr-mid**	**12**	**12**	**12**	**12**	**21**	**21**	**716**	**2.81**	**6.73**
Afrotropical migrant–Terrestrial forager–Canopy rooster	**afro-terr-can**	**6**	**6**	**18**	**18**	**44**	**9**	**138**	**2.19**	**20.44**
Paleartic migrant–Aerial forager–Canopy rooster	pal-aer-can	3	1	9	3	2	0	0	na	na
Paleartic migrant–Aquatic forager–Ground rooster	**pal-aqua-gr**	**18**	**6**	**6**	**2**	**10**	**8**	**81**	**0**	**0**
Paleartic migrant–Aquatic forager –Canopy rooster	pal-aqua-can	6	2	18	6	1	0	0	na	na
Paleartic migrant–Terrestrial forager–Ground rooster	pal-terr-gr	27	9	9	3	1	0	0	na	na
Paleartic migrant–Terrestrial forager–Mid-story rooster	pal-terr-mid	18	6	18	6	3	0	0	na	na
Paleartic migrant–Terrestrial forager–Canopy rooster	pal-terr-can	9	6	27	9	4	0	0	na	na

† Relative risks of infection by *Plasmodium* (rr.P) and *Haemoproteus* (rr.H), under two hypotheses relative to the role of movment ecology in the dynamics of avian haemosporidia: (1) H1: haemosporidia dynamics are mostly driven by exogeneous strains and (2) H2: haemosporidia dynamics are mostly driven by local strains.

‡ Number of counted (N_{spC}) and tested (N_{spT}) species.

§ Number of tested individuals.

¶ Observed *Plasmodium* (Prev.P) and *Haemoproteus* (Prev.H) prevalence.

maintenance we made the more parsimonious assumption that both functions had a similar weight.

Comparison of the counted and captured communities.—The characteristics of the "captured community" (i.e., birds we tested for malaria) were compared to those of the "counted community" (i.e., birds recorded in the point counts) for each site and globally (pooled data from all sites and times) to determine how well our sampling represented the observed bird community and quantify the bias introduced by the capture techniques and the "catchability" of the birds. The proportions of each captured species and EpiFG combination were compared to their counted proportions using Spearman's rank correlation and permutation tests.

Prevalence and avian malaria risk at the species and EpiFG combination levels.—For each species and EpiFG combination we calculated the observed prevalence of each malarial parasite as the proportion of positives among all tested birds. We

searched for differences in prevalence between sites with a chi-square test and pairwise comparisons with Holm's correction for multiple testing (Holm 1979). The effect of each behavioral trait was tested using ANOVAs on log-transformed prevalence to ensure the normality of the residuals. We tested for an effect of phylogeny on bird species' prevalence using Mantel tests to compare the interspecific differences in prevalence, measured as Bray–Curtis distances, to species phylogenetic distances, given by the tree branch lengths extracted from a subsample of the tree from (Jetz et al. 2012).

We estimated the "*a priori risk*" (*ap_risk*) of infection of species or EpiFG combination *i* by multiplying its relative risk (rr_i) by its observed proportion p_i in the total counts:

$$ap\_risk_i = rr_i \times p_i \quad (2)$$

and its "*estimated risk*" (*est_risk*) of infection by multiplying its observed prevalence ($prev_i$) by its observed proportion:

$$est\_risk_i = prev_i \times p_i. \quad (3)$$

The "a priori risks", which provide semiquantitative predictions about avian malaria circulation according to current knowledge of the disease in wild birds, and the "estimated risks", which capture the observed prevalence and the community composition, were compared using partial Spearman's rank correlations (accounting for the presence of *pi* in both risk calculation, Eqs. 2 and 3) and permutation tests at the species and EpiFG combination levels, in each ecosystem and globally. As the global prevalence of haemosporidia over all sites and studied years was 7.82%, we included only species or EpiFG combinations with ≥12 tested individuals (i.e., minimum number of individuals necessary to detect one positive). This led to the inclusion of 30 species and nine EpiFG combinations in the analysis with pooled data, and of 8–11 species and 4–5 EpiFG combinations in the analysis per site.

To identify species or combinations more or less infected than expected, we calculated the discrepancy between the two risks:

$$Di = ap\_risk_i - est\_risk_i. \quad (4)$$

All analyses were undertaken in R 3.0.1 (R Core Team 2013).

Results

Comparison of the counted and captured bird communities

Avian communities were dominated by Afrotropical migrants and nomadic birds foraging on the water and roosting on the ground (i.e., some ducks, waders, ciconiforms, and a kingfisher), by Afrotropical migrants foraging on the ground and roosting at intermediate heights (i.e., passerines, near-passerines, and Egyptian Goose), and by Afrotropical migrants foraging and roosting on the ground (i.e., gulls and waders) (Fig. 1; Appendix S2: Table S3).

The proportions of the counted and captured EpiFG combinations were significantly correlated (BAR: $\rho = 0.87$, $P < 0.001$; STR: $\rho = 0.73$, $P = 0.001$; ZIM: $\rho = 0.88$, $P < 0.001$, NGA: $\rho = 0.86$, $P < 0.001$ and globally: $\rho = 0.75$, $P < 0.001$) (Fig. 1), as were the proportions of counted and captured species, except in STR (BAR: $\rho = 0.64$, $P < 0.001$; STR: $\rho = 0.26$, $P = 0.17$; ZIM: $\rho = 0.68$, $P < 0.001$; NGA: $\rho = 0.60$, $P < 0.001$ and globally: $\rho = 0.83$, $P = 0.003$). Nonetheless, the "Afrotropical migrants-aquatic foragers–ground roosters" combination was globally over-represented in the samples tested for malaria compared to the counts (Fig. 1; Appendix S2: Table S3). The "Afrotropical migrants–terrestrial foragers–mid-story roosters" combination was under-represented in the malaria samples, especially in NGA where Red-billed Queleas were observed in large flocks but captured in fewer numbers, whereas it was over-captured in STR owing to many captures of Egyptian Geese (Fig. 1; Appendix S2: Table S3). Those discrepancies reflect the original objectives of the protocol, i.e., surveying AIV in waterbirds. Several EpiFG combinations ("res-aqua-gr", "res-terr-mid", "afro-aer-mid", "afro-aer-can", "pal-aer-can", "pal-aqua-can", "pal-terr-gr", "pal-terr-mid", "pal-terr-can") were observed but not captured, due to their scarcity at the community or global level (Fig. 1; Appendix S2: Table S3).

Observed haemosporidia prevalence

In total, 7.82% of the 3427 tested individuals were infected by haemosporidia. A total of 3414 samples could be analyzed per genus (13 samples could not be sequenced); 4.01% were infected by *Plasmodium* and 3.17% by *Haemoproteus*. ZIM had the highest *Plasmodium*

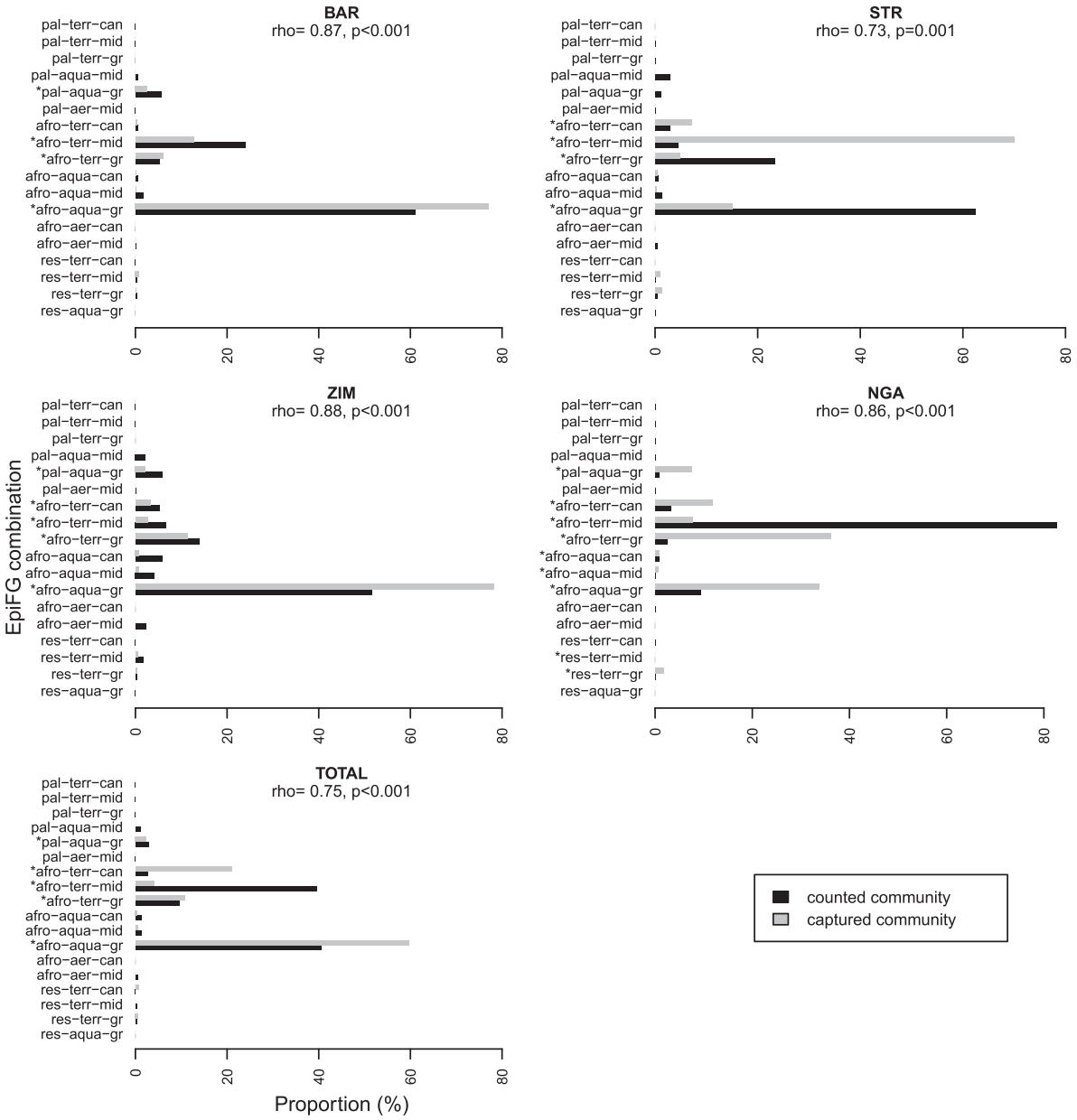

Fig. 1. Composition of the counted and captured communities at each site and in pooled data (*TOTAL*). The EpiFG combinations with ≥12 tested individuals are indicated with an asterix. The Spearman's rank correlations (ρ) between their proportions in the counted and captured communities are given with their associated *P*-value. res: resident species, afro: afrotropical migrants and nomadic species, pal: palearctic migrants, aqua: aquatic foragers, terr: terrestrial foragers, gr: ground roosters, mid: mid-story roosters, can: canopy roosters.

prevalence (6.76%), followed by NGA (2.88%), BAR (2.48%) and STR (1.63%), with significant differences between the sites (χ^2 = 44.17, df = 3, P < 0.001). Birds were significantly more infected by *Plasmodium* in ZIM than in BAR (χ^2 = 22.46, df = 1, P < 0.001) and in STR (χ^2 = 25.38, df = 1, P < 0.001). NGA had the highest *Haemoproteus* prevalence (8.93%), followed by STR (4.74%), ZIM (1.85%), and BAR (1.56%), with significant differences

between the sites ($\chi^2 = 62.60$, df = 3, $P < 0.001$). Birds were significantly more infected by *Haemoproteus* in NGA than in BAR ($\chi^2 = 43.35$, df = 1, $P < 0.001$), STR ($\chi^2 = 7.08$, df = 1, $P = 0.016$), and ZIM ($\chi^2 = 40.75$, df = 1, $P < 0.001$) and significantly more in STR than in ZIM ($\chi^2 = 12.61$, df = 1, $P = 0.001$) and BAR ($\chi^2 = 14.92$, df = 1, $P < 0.001$).

Among the 30 species with ≥12 individuals sampled, 3.74% were infected with *Plasmodium* and 2.72% with *Haemoproteus*. The highest *Plasmodium* prevalence were observed in the Helmeted Guineafowl (26.67%, $N = 15$), the Spur-winged Goose (20%, $N = 35$), and the White-faced Duck (15%, $N = 180$). The highest *Haemoproteus* prevalence were observed in the Southern Masked Weaver (66.67%, $N = 12$), the Cape Turtle Dove (36.51%, $N = 63$), and the Gray-headed Gull (31.25%, $N = 16$) (Appendix S2: Table S2). From all 86 tested species, 23 had never been recorded positive to these haemosporidia before (Appendix S1).

The *Haemoproteus* prevalence of the EpiFGs of the maintainers group was concordant with their relative "a priori risk", i.e., increasing with increasing risk (Table 1), although the prevalence was only significantly different between the EpiFGs of the maintainers via roosting height ($F = 4.77$, $P = 0.02$; Fig. 2b). For *Plasmodium*, the prevalence and the relative "a priori risks"of the maintainers group were not concordant neither via the foraging substratum, nor via the roosting height (Table 1). The latter had a significant effect on *Plasmodium* prevalence ($F = 3.47$, $P = 0.05$), but with the mid-story roosters being more infected than the ground and canopy roosters (Fig. 2e). For the introducers group, the "a priori risks" were concordant with the prevalence of both genera under H2 (dynamics driven by local strains) but not H1 (dynamics driven by exogeneous strains) (Table 1). Resident birds were significantly more infected by *Plasmodium* than nomadic or migratory species ($F = 4.25$, $P = 0.025$, Fig. 2a), but the difference was not significant for *Haemoproteus* ($F = 1.50$, $P = 0.24$, Fig. 2b). In addition, birds' phylogenetic distances were significantly correlated with interspecific differences in *Plasmodium* prevalence ($R = 0.22$, $P = 0.005$) and close to being significantly correlated with those in *Haemoproteus* prevalence ($R = 0.09$, $P = 0.06$).

From the 18 EpiFG combinations represented in the counts, representatives of nine were both captured and had ≥12 sampled individuals. Resident species foraging on the ground and roosting at the mid-story or ground level were the most infected by *Plasmodium* globally (26.32%, $N = 23$ and 23.81%, $N = 21$, respectively). Afrotropical species foraging on the ground and roosting at the canopy level were the most infected by *Haemoproteus* (20.44%, $N = 138$) (Table 2). The prevalence of all combinations at each site is given in Appendix S2: Table S3.

Comparison of the "a priori" and "estimated" risks

Haemosporidia "a priori" and "estimated" risks at the species level.—We calculated the "a priori" and "estimated" risks using the relative abundance data from the counts (Eqs. 2 and 3). For the 30 species with ≥12 sampled individuals, and under the hypothesis that haemosporidia dynamics are driven by exogeneous strains (H1), the two types of risk were not significantly correlated for either *Plasmodium* ($\rho = -0.24$, $P = 0.33$), or *Haemoproteus* ($\rho = 0.25$, $P = 0.33$). By site, the partial correlation was significant for *Haemoproteus* in STR ($\rho = 0.61$, $P < 0.001$) but was not significant for the other parasite-site combinations (Appendix S2: Table S4). Under the hypothesis that infections are driven by local strains (H2), the "a priori" and "estimated" risks were significantly positively correlated for *Haemoproteus* ($\rho = 0.90$, $P < 0.001$) but not for *Plasmodium* ($\rho = 0.24$, $P = 0.33$). By site, the partial correlation was significant for BAR ($\rho = 0.52$, $P < 0.001$) and STR ($\rho = 0.61$, $P < 0.001$) but was not significant for the other parasite-site combinations, due to a lack of power (Appendix S2: Table S4). Our a priori classification thus predicts *Haemoproteus* prevalence if we consider that resident birds are more exposed to the parasite but does not predict *Plasmodium* patterns, regardless of the hypothesis used to infer the influence of bird movement.

Comparing the "a priori" risks calculated under H2 and the observed risks revealed that the "a priori" risk of *Plasmodium* infection was particularly overestimated ($D_i > \text{mean}(D_i) + sd(D_i)$) in the Red-Knobbed Coot (Fig. 3a). The "a priori" risk of *Haemoproteus* infection was particularly overestimated in the Red-billed Quelea and the Red-Knobbed Coot (Fig. 3c) and underestimated ($D_i < \text{mean}(D_i) + sd(D_i)$) in the Gray-headed Gull (Fig. 3c).

Haemosporidia "a priori" and "estimated" risks at the EpiFG combination level.—When considering

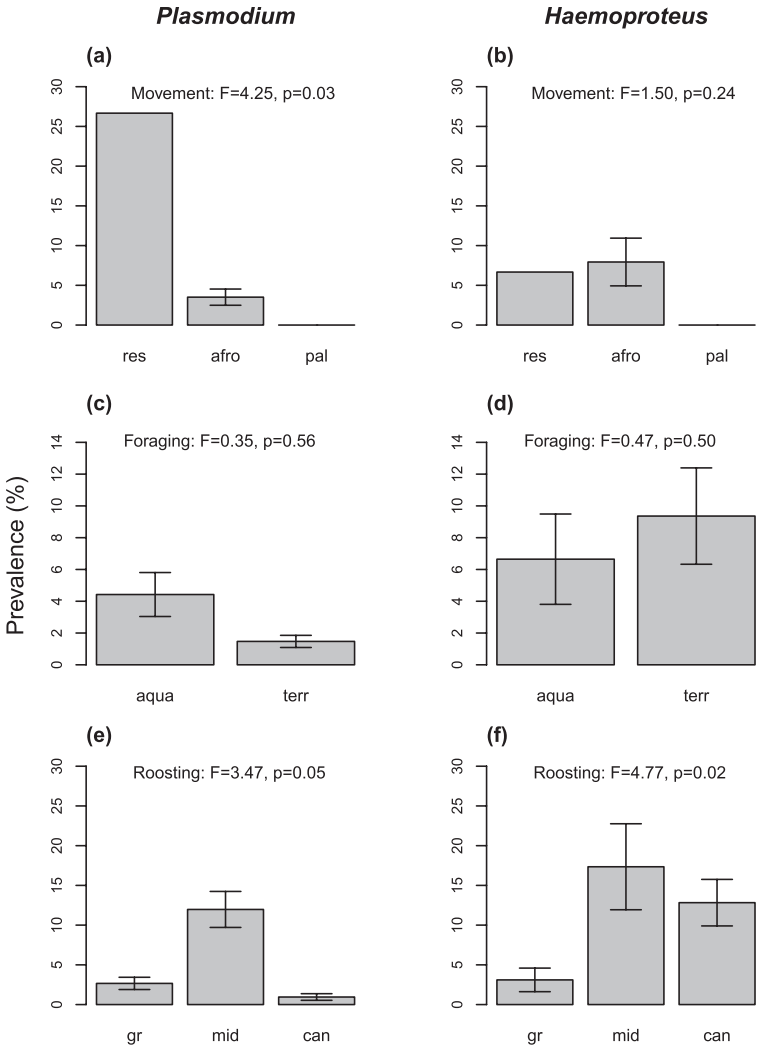

Fig. 2. Average (±standard error) *Plasmodium* (a, c and e) and *Haemoproteus* (b, d and f) prevalence in each EpiFG. res: resident species, afro: afrotropical migrants and nomadic species, pal: palearctic migrants, aqua: aquatic foragers, terr: terrestrial foragers, gr: ground roosters, mid: mid-story roosters, can: canopy roosters.

infections as driven by exogeneous strains (H1), the "a priori" and "estimated" risks of the nine EpiFG combinations were not significantly correlated for any of the malaria genera across communities (*Plasmodium*: $\rho = -0.05$, $P = 0.34$; *Haemoproteus:* $\rho = 0.90$, $P = 0.33$) or within communities, except for *Haemoproteus* in BAR ($\rho = 0.89$, $P < 0.001$) (Appendix S2: Table S4). When considering infections to be driven by local strains (H2), however, the two risks were significantly positively correlated accross communities for *Haemoproteus* but not for *Plasmodium* ($\rho = 0.80$, $P = 0.001$ and $\rho = 0.43$, $P = 0.25$, respectively). Within communities, the correlation was significant for *Haemoproteus* at BAR ($\rho = 0.94$, $P < 0.001$) but was not significant or not testable due to a lack of power for the other parasite-site combinations (Appendix S2: Table S4).

Calculating the discrepancy between "a priori" and observed risks revealed that we particularly overestimated the "a priori" risk of both *Plasmodium* and *Haemproteus* infection in the afrotropical migrants foraging on the ground

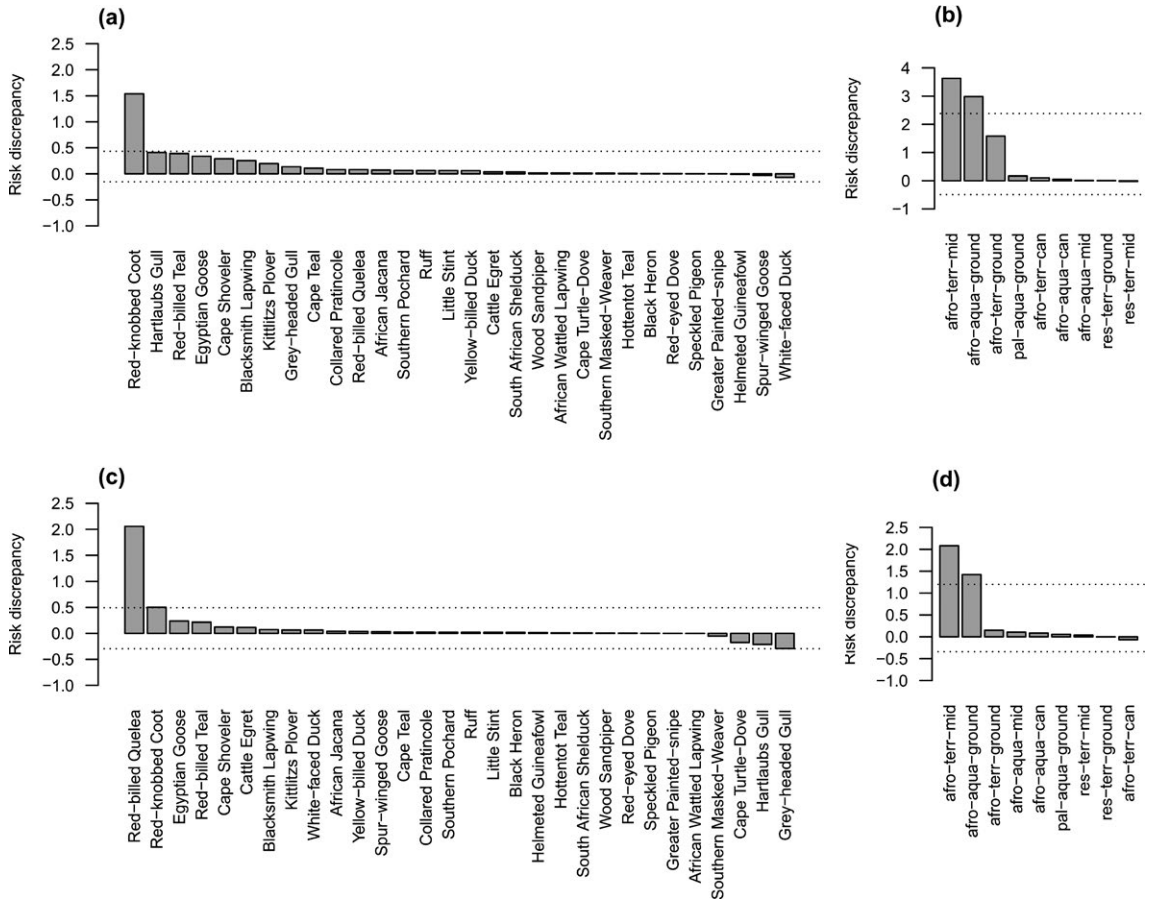

Fig. 3. Discrepancy between a priori and observed risks for *Plasmodium* (a, b) and *Haemoproteus* (c, d), at the species (a, c) and EpiFG combination (b, d) level. The a priori risks were calculated here under hypothesis H2 regarding the influence of bird movement ecology, i.e., haemosporidia dynamics are driven by local strains. The dashed lines show the thresholds for an a priori risk particularly overestimated (D_i > mean(D_i) + $sd(D_i)$) or underestimated (D_i < mean(D_i) + $sd(D_i)$). res: resident species, afro: afrotropical migrants and nomadic species, pal: palearctic migrants, aqua: aquatic foragers, terr: terrestrial foragers, gr: ground roosters, mid: mid-story roosters, can: canopy roosters.

or on the water and roosting at the mid-story or ground level (Fig. 3b,d).

Discussion

We tested the hypothesis that movement, foraging and roosting ecology are important drivers of malaria infection risk mainly because of their influence on avian exposure to malaria vectors. Our results indicate that this hypothesis is supported for *Haemoproteus*, for which we could predict infection patterns using an a priori classification of hosts in four quite different bird communities in southern Africa. This hypothesis was however rejected for *Plasmodium*, for which patterns could not be predicted.

Although our approach only yielded a predictive tool for *Haemoproteus*, it did provide some useful insights for both parasites. Some of the ecological traits that we measured appear to influence infection risk, although not always in the expected manner. Roosting height was found to have a significant effect on the prevalence of both malarial parasites, suggesting that the vertical distribution of vectors influences birds' exposure to infection. Bird species roosting at the mid-story level were more exposed to *Plasmodium* than species roosting on the ground or at the canopy

level, while *Haemoproteus* was more prevalent in birds roosting at the canopy level and at the mid-story level compared to those roosting on the ground. These differences between the two parasite genera were expected based on the different vertical distribution of their respective vectors. *Plasmodium* was nonetheless expected to be more prevalent on the ground (Mellor et al. 2000, Sinka et al. 2010). On the contrary, foraging substratum (tested for the first time as a potential risk factor for avian malaria) did not have a significant effect on avian infection risk, suggesting that the vertical but not the horizontal distribution of vectors drives birds' exposure to avian haemosporidia.

Nest height has previously been found to be positively associated with haemosporidia prevalence (Garvin and Remsen 1997, Fecchio et al. 2011). As breeding birds spend a good amount of time at the nest during both day and night, the correlation probably results from a high nocturnal exposure to host-seeking vectors, which is concordant with the higher *Haemoproteus* prevalence in mid-story and canopy roosters observed in this study as biting midges are thought to seek hosts higher up (Garvin and Greiner 2003, Cerný et al. 2011). Little is known about avian malaria vectors in southern Africa and studies of the vertical and horizontal distribution of mosquitoes and biting midges in the region would be valuable.

The movement ecology of the birds significantly influenced *Plasmodium,* but not *Haemoproteus* prevalence. However, calculating the "a priori" risks under the hypothesis that resident birds were more exposed (H2) rendered the correlation with *Haemoproteus* observed risks significant and increased the correlation for *Plasmodium*, though not significantly. This suggests that haemosporidia dynamics are more driven by local strains than by exogeneous strains, with resident species more exposed to parasites than migratory birds. A lower prevalence of avian malaria in long-distance migrants was observed previously (e.g., Hellgren et al. 2007, Pardal et al. 2013), with explanations ranging from a lower exposure to the parasite in their original populations, a greater investment in immunity due to selection experienced in their breeding and wintering areas (Møller and Erritzoe 1998) to the death of infected birds during migration (i.e., migration filter). As we do not know which strains are infecting migratory and resident birds and whether they differ, we cannot say if the low prevalence in migrants implies a low introduction risk of exogenous strains in our systems and/or if migrants can spread local strains to their breeding populations (Waldenström et al. 2002, Valkiunas 2005). This would require sequencing the circulating strains and knowing the arrival and infection time of migratory birds.

Our results identify species that may potentially be maintenance hosts of avian malaria in southern Africa. Although maintenance hosts are difficult to identify in the field (Viana et al. 2014), we can expect hosts with a high prevalence and highly exposed to vectors to have a key role in the maintenance of the parasite in the system. From our well-sampled hosts, five species meet both criteria for *Plasmodium* (ground or mid-story roosters with a prevalence over 8%): the Helmeted Guineafowl, the Red-billed Quelea, and three duck species: the Spur-winged Goose, the White-faced Duck, and the Hottentot Teal. For *Haemoproteus,* four passerine species may be maintenance hosts (canopy or mid-story roosters with a prevalence over 8%): the Southern Masked Weaver, and three Columbiforms: the Cape Turtle Dove, the Red-eyed Dove, and the Speckled Pigeon (Appendix S1). All these species have never or very rarely been considered in the study of avian malaria (see references in Appendix S1). Hosts with a very low prevalence that are rarely in contact with vectors may by contrast be dead-ends for the parasites, i.e., susceptible but participating very little in parasite transmission. For *Haemoproteus* this may be the case for two ducks: the Red-billed Teal and the Yellow-billed Duck and for the Red-knobbed Coot (Rallidae) (Appendix S1). For *Plasmodium*, no species meet both criteria; the Red-knobbed Coot was very poorly infected by this genus but has the potential to participate more in its transmission due to its high exposure to mosquitoes while roosting on the ground.

The patterns of infection observed in this study and in particular the discrepancies between the expected and observed patterns for certain species (the Red-knobbed Coot, the Red-billed Quelea, and the Gray-headed Gull) and EpiFG combinations (afrotropical migrants, foraging on the ground or on the water and roosting at the mid-story or ground level) may also result from other

unconsidered factors. First, a variety of ecological characteristics may drive host exposure in our system. Vector contact rates with hosts are often a function of host abundance (Begon et al. 2009). We included the relative abundance of bird species in risk calculations; however, this had to be corrected for when estimating the correlation between the expected and observed risks. Although our data set did not allow us to rigorously test the hypothesis of an effect of host relative abundance on prevalence at each sampling period, a post hoc test revealed that it may influence positively *Plasmodium* prevalence globally (Spearman's correlation: $\rho = 0.38$, $P = 0.02$), but not *Haemoproteus* ($\rho = -0.08$, $P = 0.35$). The discrepancy between the expected and observed risks of infection may also result from (1) vector feeding preferences: even the generalist mosquitoes such as *C. pipiens* can over- or under-use some bird species (Hamer et al. 2009), which can in turn influence their transmission of diseases (Simpson et al. 2012); (2) different bird defense strategies: bird species may vary in their capacity to limit or prevent vectors from obtaining a blood meal due to differences in physical (feathers, scales) and behavioral (dexterity to remove mosquitoes, shielding exposed areas) adaptations (Darbro and Harrington 2007); (3) nest type or sociality: open-cup nesters have been shown to be more infected than cavity and dome nesters, as were some group-living birds or cooperative breeders (Fecchio et al. 2011). However, species with an a priori risk particularly over- or under-estimated all had open nests and were gregarious, suggesting no influence of these factors.

Second, interspecific variation in prevalence may result from immunological, physiological or genetic variations, as observed for avian influenza in wildfowl (Gaidet et al. 2012). Variations in host susceptibility due to differences in host–parasite co-evolution histories or host traits, may explain differences in prevalence (Medeiros et al. 2013). These were not included in the EpiFGs because current knowledge of the bird-malaria system is insufficient for us to translate phylogenetic distances into competence scores. However, we showed that the observed interspecific differences in *Plasmodium* prevalence were significantly correlated with birds' phylogenetic distances. Also, the potential maintenance hosts identified for each parasite were not random but included three duck species for *Plasmodium* and three Columbiforms for *Haemoproteus*.

Although some studies have considered avian haemosporidia across numerous families of passerines (Ricklefs et al. 2005, Hellgren et al. 2009, Svensson-Coelho et al. 2013, Okanga et al. 2014), very few have considered the diversity of birds included here, including waterbirds and galliformes. The overall prevalence observed in our communities (3.74% for *Plasmodium* and 2.72% for *Haemoproteus* in the samples with ≥12 individuals tested) were low compared to other studies, especially for *Haemoproteus,* for which a prevalence over 10% is regularly recorded (Bennett et al. 1992, Loiseau et al. 2010), including in southern Africa (Schultz et al. 2011, Cumming et al. 2013). Explaining this low prevalence would require investigating many biotic (e.g., vector and host diversity and abundance) and abiotic (e.g., rainfalls, temperatures, elevation) factors and was not the purpose of this study. We can nonetheless note that Passeriformes exhibited a high prevalence of both genera in our study (11.11% and 26.39% for *Plasmodium* and *Haemoproteus*, respectively), validating our sampling protocol, while the more intensively-sampled waterbirds were much less infected (2.94% by *Plasmodium*, 1.77% by *Haemoproteus*), possibly due to the ducks' dense and oily plumage.

As a whole, *Plasmodium* prevalence seemed to be influenced by the movement ecology, roosting height, and the relative abundance and the phylogeny of the bird species; while *Haemoproteus* transmission seemed to be mostly driven by the roosting height of its avian hosts. Even for parasites that are relatively generalist, explaining infection patterns at the host community level requires considering both factors influencing host exposure (e.g., behavioral factors) and factors influencing host susceptibility (e.g., phylogeny, physiology). The inclusion of all these factors in our a priori classification was not possible due to the current state of knowledge. EpiFGs were nonetheless sufficient to predict *Haemoproteus* patterns in our bird communities and it should be possible to use our approach to summarize bird communities in a meaningful way for *Plasmodium* epidemiology once the system is better understood.

Being able to predict infection patterns using such an a priori classification will enable using

host community composition and abundance data to draw conclusions on disease maintenance and introduction risk, without running expensive and demanding host captures and sampling. We believe that EpiFGs therefore offer many new and potentially exciting opportunities in epidemiology and ecosystem studies, for instance to track in time the relationship between changes in host assemblages and disease risk (Caron et al. 2010) or to anticipate and predict the consequences for disease risk of changes in host communities' composition due to anthropogenic activities or climate change (Lambin et al. 2010).

Conclusion

Globally, our findings show that even in a system in which the parasites are relatively generalist, the likelihood of infection with avian malaria is driven by a mix of ecological and evolutionary factors. Although some of the ecological traits chosen for their expected epidemiological relevance (e.g., roosting and movement behavior) seem to have an influence on birds' infection risk, host taxonomy and/or compatibility factors at the host–parasite interface also play a role in driving hosts–parasites contacts and must be accounted for to understand infection levels at the community level. Our results confirm that accounting for the composition of the host community and the ecology and taxonomy of its members, is crucial for understanding the dynamics of multihost parasites.

Although using EpiFGs as a predictive tool in our system still requires some developments, in particular to predict *Plasmodium* patterns, EpiFGs offer a new conceptual tool to consider multihost parasites in host communitites. Just as research on ecological functional groups has facilitated advances in community ecology, EpiFGs have the potential to help researchers cope with the theoretical and management challenges raised by multihost parasites and offer opportunities to further explore the complex relationship between disease and biodiversity (Caron et al. 2015).

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