

Host and habitat specialization of avian malaria in Africa

CLAIRE LOISEAU,*†RYAN J. HARRIGAN,†‡ALEXANDRE ROBERT,§ RAURIC. K. BOWIE,¶**
HENRI A. THOMASSEN,†‡THOMAS B. SMITH†‡and RAVINDER N. M. SEHGAL*†

*Department of Biology, San Francisco State University, 1600 Holloway Avenue, San Francisco, CA 94132, USA, †Center for Tropical Research, Institute of the Environment, UCLA, Los Angeles, CA 90095-1606, USA, ‡Department of Ecology and Evolution, University of California, Los Angeles, CA 90095 USA, §Conservation des Espèces, Restauration et Suivi des Populations, UMR 7204 MNHN-CNRS-UPMC, 55 rue Buffon, 75005 Paris, France, ¶Museum of Vertebrate Zoology and Department of Integrative Biology, 3101 Valley Life Science Building, University of California, Berkeley CA 94720, USA, **Percy FitzPatrick Institut, DST/NRF Centre of Excellence, University of Cape Town, Rondebosch 7701, South Africa

Abstract

Studies of both vertebrates and invertebrates have suggested that specialists, as compared to generalists, are likely to suffer more serious declines in response to environmental change. Less is known about the effects of environmental conditions on specialist versus generalist parasites. Here, we study the evolutionary strategies of malaria parasites (*Plasmodium* spp.) among different bird host communities. We determined the parasite diversity and prevalence of avian malaria in three bird communities in the lowland forests in Cameroon, highland forests in East Africa and fynbos in South Africa. We calculated the host specificity index of parasites to examine the range of hosts parasitized as a function of the habitat and investigated the phylogenetic relationships of parasites. First, using phylogenetic and ancestral reconstruction analyses, we found an evolutionary tendency for generalist malaria parasites to become specialists. The transition rate at which generalists become specialists was nearly four times as great as the rate at which specialists become generalists. We also found more specialist parasites and greater parasite diversity in African lowland rainforests as compared to the more climatically variable habitats of the fynbos and the highland forests. Thus, with environmental changes, we anticipate a change in the distribution of both specialist and generalist parasites with potential impacts on bird communities.

Keywords: avian malaria, generalist, habitat specificity, *Plasmodium*, specialist

Received 25 December 2010; revision received 13 September 2011; accepted 29 September 2011

Introduction

The existence of both generalist and specialist species raises several questions: why have these strategies evolved, and for a given set of environmental conditions, is there an optimal strategy? Much attention has been paid to how species distributions and populations might be affected by environmental change (Pounds *et al.* 1999; Both & te Marvelde 2007; Gordo 2007; Hitch & Leberg 2007). In addition, the majority of research on specialization has been performed on both vertebrates

and invertebrates, with most studies suggesting that niche specialists have suffered extensive declines as compared to generalists (insects: Hughes *et al.* 2000; Kotze & O'Hara 2003; reptiles: Foufopoulos & Ives 1999; fish: Munday 2004; birds: Owens & Bennett 2000; Julliard *et al.* 2004; mammals: Harcourt *et al.* 2002; Fisher *et al.* 2003), and that specialists are more likely to go extinct when faced with global environmental changes (McKinney 1997; Devictor *et al.* 2008; Colles *et al.* 2009; Clavel *et al.* 2010; Ekroos *et al.* 2010).

However, it remains unclear how habitat and environmental changes will impact parasites (Mostowy & Engelstaedter 2011). In contrast to their hosts, the environment of endoparasites is composed of at least two

Correspondence: Claire Loiseau, Fax: 415 338 2295;
E-mail: cloiseau@sfsu.edu

ecologically different dimensions: the host and the environment of the host (Thomas *et al.* 2002). In addition, in the case of non-free-living forms, such as vector-borne parasites, the modification of vector environment is a pivotal component. Therefore, environmental changes, such as habitat fragmentation and global climate change, can influence the evolutionary trajectories of parasites by affecting interactions between the pathogen and the arthropod vector, the host, or a combination of both (Zell 2004; Brooks & Hoberg 2008; Lafferty 2009; Sehgal 2010; Tabachnick 2010; Massad *et al.* 2011).

Malaria parasites are among the most intensively investigated vector-borne parasites and a major cause of human mortality (WHO 2009). In addition, they have been studied extensively in birds because of their diversity of hosts and nearly worldwide distribution (Valkiūnas 2005). Effects of habitat characteristics and climate on avian *Plasmodium* prevalence have been investigated at various geographical scales (Merilä *et al.* 1995; Bensch & Åkesson 2003; Wood *et al.* 2007; Svensson & Ricklefs 2009; Bonneaud *et al.* 2009; Chasar *et al.* 2009; Ishtiaq *et al.* 2010; Sehgal *et al.* 2011; Knowles *et al.* 2010; Garamszegi 2011). Also to our knowledge, studies have focused on the effects of environmental variables on the host specificity of parasites (Poulin *et al.* 2011), but it is the first time regarding avian malaria parasites. To understand whether generalist or specialist vector-borne parasites are likely to have a competitive advantage under global environmental change, studies must take into account not only the environmental conditions under which these parasites exist but also the geographical distribution of their hosts, parameters themselves linked to the habitat.

The distinction between specialist and generalist categories can be perplexing; Hellgren *et al.* (2009) demonstrated that parasites with the ability to successfully infect a wide variety of host species can also be the most prevalent in a single host species. Specialist and generalist strategies might be best thought of as extremes along a continuum. As an example, evolutionary relationships of avian blood parasites have revealed that several lineages of *Plasmodium* exhibit extreme generalist host-parasitism strategies, whereas other lineages appear to have been constrained to certain host families, or individual species (Ricklefs & Fallon 2002; Ricklefs *et al.* 2004; Beadell *et al.* 2009). It has been suggested that specialization represents an evolutionary 'dead-end', which limits further evolution (Kelley & Farrell 1998; Snyder & Loker 2000; Nosil 2002). However, several studies have indicated that generalists can be repeatedly evolved from specialist lineages (Poulin *et al.* 2006; Johnson *et al.* 2009; Gomez *et al.* 2010).

Because of infections with a high diversity of both specialist and generalist parasites, African passerine

birds provide an excellent model system to test hypotheses about the evolutionary strategies of generalist vs. specialist parasites (Beadell *et al.* 2009). To date, however, most of the studies on avian malaria in Africa have been carried out in West and Central Africa (Kirkpatrick & Smith 1988; Waldenström *et al.* 2002; Sehgal *et al.* 2005; Hellgren *et al.* 2007; Beadell *et al.* 2009; Bonneaud *et al.* 2009; Chasar *et al.* 2009; Loiseau *et al.* 2010). Less is known about the diversity and prevalence of these parasites in other regions of Africa. Threatened habitats in East Africa, such as highland forests (Hall *et al.* 2009; Giam *et al.* 2010) and the fynbos (a natural Mediterranean shrubland vegetation occurring in a small belt along the west and south coasts of South Africa; Midgley *et al.* 2002; Fairbanks *et al.* 2004), are particularly interesting, because they exhibit more variable environmental conditions in terms of temperature and rainfall than lowland rainforests (Bodker *et al.* 2003). We set out to compare these habitats (lowland, highland and fynbos) because of their distinct environmental characteristics that contribute to different communities of birds and vectors with varying degrees of specialization within their habitats (De Klerk *et al.* 2002). In the near future, the communities of birds in these habitats will likely suffer declines because of rapid environmental changes (Berg *et al.* 2010).

We predict that habitats subject to highly temporally variable environmental conditions, as exemplified by montane highlands or the fynbos, will have a greater impact on the environment of the vectors, as they are temperature sensitive (Paaijmans *et al.* 2009; Tonnang *et al.* 2010) and the community of birds should harbour more generalist parasites (i.e. more adaptable) than the lowlands, where the climate is more constant throughout the year (i.e. more prone to specialization). Therefore, for each of the three habitats, the objectives of this study were to: (i) determine the parasite diversity and prevalence of avian malaria, (ii) determine the host and habitat specificity and (iii) examine the parasites phylogenetic relationships, particularly with regard to the transition rates between generalist and specialist states. Understanding how current habitat variability affects the host specificity of pathogens and how strategies have changed during the evolution of this parasite group will aid in predicting optimal adaptive strategies under rapid global environmental change.

Methods

Sample sites

Blood samples were collected in three distinct habitats across the African continent (36 sites; coordinates given in Table S1 and distances between sites in

Tables S6–S8; see also map in Fig. S1, Supporting information). The characteristics of each site were determined using a set of environmental variables that included bioclimatic metrics from the WorldClim data set (Hijmans *et al.* 2005), <http://www.worldclim.org>, Normalized Difference Vegetation Index (Huete *et al.* 2006), the percentage of tree cover (Hansen *et al.* 2002) and elevation data from the Shuttle Radar Topography Mission (see Appendix S1 part A, for description of the bioclimatic and habitat variables; Sehgal *et al.* 2011). Bioclimatic metrics from the WorldClim data set showed that lowland forest sites experience high annual mean temperature, low temperature seasonality, high annual precipitation and moderate precipitation seasonality. Highland sites are characterized by moderate annual mean temperatures, temperature seasonality and moderate annual precipitation, but with high precipitation seasonality. Fynbos sites experience relatively low annual mean temperatures, high temperature seasonality, low annual precipitation and moderate precipitation seasonality.

We captured eight bird species from five families among the nine West African lowland forest sites ($N = 575$; Table S2, supporting information), 11 bird species from five families at 17 sites in East African

highland forests ($N = 406$; Table S3) and six bird species from four families at 10 sites in South Africa in the fynbos ($N = 383$; Table S4, Supporting information). We found *Plasmodium* spp. infections in 8, 5 and 12 sites, respectively (Table 1). Two families of birds are represented in all three habitats, Nectariniidae (sunbirds) and Pycnonotidae (bulbuls), and two further families are present in both West and East Africa, Turdidae (thrushes) and Muscicapidae (Old World flycatchers). Each location was sampled using mist nets. Blood samples were collected from the brachial vein and stored in lysis buffer (10 mM Tris-HCL pH 8.0, 100 mM EDTA, 2% SDS) or in dimethyl sulfoxide.

Parasite screening

DNA was extracted from whole blood following a DNeasy kit protocol (Qiagen, Valencia, CA, USA). Successful DNA extraction was verified with primers that amplify the brain-derived neurotrophic factor (Sehgal & Lovette 2003). For *Plasmodium* detection, we used a nested PCR to amplify a fragment of cytochrome *b* (524 bp) with the primers HAEMF/HAEMR2 – HAEMNF/HAEMNR2 following Waldenström *et al.* (2004). We included positive controls using samples with

Table 1 Numbers of screened and infected individuals as well as the number of *Plasmodium* lineages are given per site in each habitat

Habitat	Site	N Individuals	N Infected	N Lineages	Prevalence (%)
Lowland	Ngoila	93	30	8	32.3
	Zoebefame	93	32	11	34.4
	Bobo Camp	96	40	9	41.7
	Douni	39	5	5	12.8
	Mvono	25	5	2	20.0
	Beh	48	13	5	27.1
	Mvia	45	18	6	40.0
	Mokoko	63	19	9	30.2
Fynbos	Beaufort West	4	1	1	25.0
	Jonkershoek	256	25	5	9.8
	Koeberg	4	1	1	25.0
	Cape peninsula	18	1	1	5.6
	Bontebok NP	6	5	1	83.3
Highland	Kilimanjaro	2	1	1	50.0
	West Usambara	43	1	1	2.3
	East Usambara	19	4	2	21.1
	Nguru	56	13	5	23.2
	Rubeho	48	9	3	18.8
	Uluguru	29	3	2	10.3
	Udzungwa	41	10	3	24.4
	Livingstone	18	1	1	5.6
	Mahenge	7	1	1	14.3
	Misuku	11	1	1	9.1
	Nyika	18	1	1	5.6
Namuli	15	3	2	20.0	

known infections as evident from microscopy results (Valkiūnas *et al.* 2008a), as well as negative controls using purified water in place of DNA template. The PCR products were run out on a 2% agarose gel using 1× TBE and visualized by ethidium bromide staining under ultraviolet light to check for positive infections. PCR products were purified using ExoSap (following the manufacturer's instructions, USB Corporation, Cleveland, OH, USA). We identified lineages by sequencing the fragments (BigDye [R] version 3.1 sequencing kit; Applied Biosystems) on an ABI PRISM 3100 (TM) automated sequencer (Applied Biosystems). In some cases, co-infection is not detected by PCR; however, Valkiūnas *et al.* (2008b) demonstrated that both the PCR and microscopy work showed similar prevalence.

Habitat classification

To ensure that the spatial pattern of the selected sample sites was not responsible for the results obtained for the difference in prevalence, we tested for spatial autocorrelations (Appendix S1, part B). In addition, to determine whether differences in environmental variables could accurately classify each habitat type, we constructed classification trees using a random forest model (RandomForest; Liaw & Wiener 2002) in R (R Development Core Team: R 2004), with climate variables (extracted for each site) as predictors and habitat type as a response variable.

Under a random forest classification model, climate variables correctly classified 97% of sites (35/36, classification error = 0.0588) as belonging to one of the three habitat types (lowland forest, fynbos, highland forest). The variables most important in determining these classifications were temperature seasonality (BIO4) and minimum temperature of the coldest month (BIO6). These results indicate that each of the three habitats can be distinguished from one another solely by environmental characteristics, particularly ground-based bioclimatic measurements.

Parasite prevalence and distribution

Potential differences in parasite prevalence between habitats were tested using generalized linear mixed-effects models (hereafter, GLMM) with binomial distribution of errors and logit link function. The dependent variable was the proportion of infected birds in each site (weighted by the number of individuals sampled), and explanatory variables were the habitat type (factor, fixed effect variable), parasite lineage and site (factors, random effect variables; assuming that site was nested within habitat). The host–parasite assembly rule was

investigated using *ad hoc* randomization tests. Based on the prevalence of parasites in each habitat (regardless of host species), the abundance of birds of all species (estimated by sample sizes), and assuming that host–parasite associations are random, a theoretical distribution of the number of species infected by each lineage in each habitat was constructed (null model). Actual numbers of infected species were then compared with these expectations (details presented in Appendix S1, part C).

Host specificity index

Host specificity of the parasite lineages was measured as the number of host species in which parasites were found. To obtain a measure that included both the diversity of host species and the taxonomic distance between these species, we used the modified version of the host specificity index S_{TD} (Poulin & Mouillot 2003; Høllgren *et al.* 2009). Therefore, a host specificity index has been calculated for each parasite lineage, with high values representing generalist parasites and low values specialist parasites. We calculated (i) the host specificity index with only our data, i.e. local specificity index and (ii) the host specificity index with the data available in Genbank and MALAVI database (Bensch *et al.* 2009), i.e. global specificity index. Indeed, we found lineages previously discovered at different locations in different host species, and we combined all available data to estimate a global host specificity index. Host specificity indices are given in Table S5 (supporting information).

For each site in each habitat, S_{TD} index for a parasite lineage was weighted by the percentage of individuals infected by this parasite lineage to take into account the number of times that a parasite has been found. Potential differences in S_{TD} between habitats were tested using both linear (hereafter, LM) and linear mixed-effects models (LMM). In both types of models, dependent variables were the local and global S_{TD} in each site (log transformed). In LM, the habitat type was the only explanatory variable. In linear mixed-effects models, explanatory variables were the habitat type (factor, fixed effect variable), parasite lineage and site (factors, random effects variables; assuming that site was nested in habitat). S_{TD} values were weighted by sample sizes in each site to correct for heterogeneity in sampling variance. LM was used to test for global differences in parasites' host specificity between habitats (pairwise differences between habitats were subsequently tested using *post hoc* Tukey test). Linear mixed-effects models was used to assess whether particular parasite lineages show differences in S_{TD} in the different habitats. GLM, linear mixed-effects models and GLMM models were fitted in the R 2.10.1 framework (R Development Core

Team 2009), specifically using the LME4 package (Crawley 2007).

In addition, we performed a mixed model to test whether the global host specificity indices of parasites were associated with the geographical range of their host (as a repeated variable). The geographical range was determined using data available from BirdLife International (<http://www.birdlife.org/index.html>) and was used as a fixed factor in the model, and the host species was used as a random factor (SAS 1999).

Phylogenetic analyses and evolutionary relationships

Sequences obtained were aligned using Sequencher 4.8 (GeneCodes, Ann Arbor, MI, USA). A phylogenetic tree was constructed using 34 mitochondrial cytochrome *b* sequences of avian *Plasmodium* spp. We compared these sequences to all sequences from

Plasmodium lineages previously deposited in Genbank and the MALAVI database (<http://mbioserv4.mbioeokol.lu.se/avianmalaria/index.html>; Bensch *et al.* 2009). Our sequencing analyses also identified birds infected with *Haemoproteus* lineages; although not used in these analyses, prevalence and lineage data of these parasites in these populations are available upon request. All unique sequences were verified via duplicate sequencing. GenBank accession numbers are shown in Fig. 1. All individual sequences were grouped into a consensus that was 500 bp long, with *P. gallinaceum* (GenBank accession number NC008288) as the outgroup. We first determined the model of sequence evolution that best fit the data using MRMODELTEST (Nylander 2004). Bayesian analysis of the sequence data was then conducted with MRBAYES version 3.1.2 (Huelsenbeck & Ronquist 2001) using the model of sequence evolution obtained from MRMODELTEST

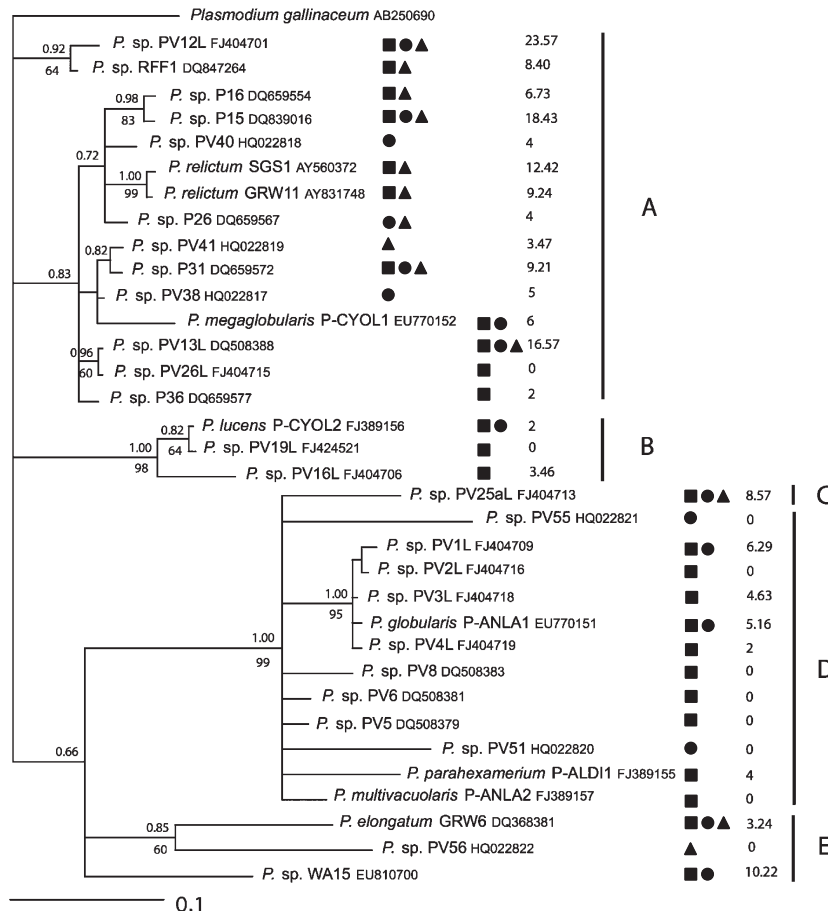


Fig. 1 Phylogenetic relationships in a consensus tree of the 34 *Plasmodium* lineages found in the three communities of birds based on cytochrome *b* sequences. *Plasmodium gallinaceum* was used as the outgroup. GenBank accession numbers of all sequences are indicated. Numbers located on the top of the branches indicate Bayesian probability values, and below, ML bootstrap support (100 replications, only values above 50% are shown). Symbols depict the different habitats (square: lowland rainforest; circle: highland forest; triangle: fynbos). The S_{TD} index is given for each lineage. The bars indicate three groups of parasites (A: host generalist; B: host specialist; C: host specialist).

(GTR + G + I). Two Markov chains were run simultaneously for 10 million generations and sampled every 200 generations, for a total of 50 000 trees each, sampled from the posterior distribution. For the 'burn-in', 25% of the trees were discarded from the posterior distribution. The remaining trees were used to calculate the posterior probabilities. Phylogenetic analyses were also implemented using maximum likelihood in PAUP* 4.0 (Swofford 2003). The support for the individual branches was estimated using maximum likelihood bootstrap analyses with 100 replicates. The topology of both the Bayesian and the maximum likelihood trees was almost identical, with only small differences in some branches that lacked significant support from either method.

In addition, we performed ancestral state reconstruction analysis under a maximum-likelihood framework to estimate the states of strategies (generalist vs. specialist) on phylogenies using BayesTraits (Pagel 1999; <http://www.evolution.rdg.ac.uk>). Using the consensus tree obtained from phylogenetic analyses and the S_{TD} values of sampled branches, we performed ancestral character state reconstructions at each node of the consensus tree. Likelihood scores were acquired from BayesTraits and tested (via likelihood ratio tests) for a model that did not control rates of change between states (generalist to specialist and *vice versa*), as compared to a model that forced these rates to be equal (Appendix S1, part D).

Results

Parasite prevalence, diversity and distribution

In total, from 25 bird species (1364 individuals), we found 34 *Plasmodium* lineages. In the lowland rainforest habitat, we screened 575 individuals, of which 177 were infected with a total of 18 *Plasmodium* lineages (Table S2). In the highland forest, 406 individuals were screened, of which 51 were infected with a total of 13 *Plasmodium* lineages (Table S3). In the fynbos habitat, we screened 383 individuals, of which 51 were infected with a total of 9 *Plasmodium* parasite lineages (Table S4, supporting information).

Several lines of evidence suggested that parasites were not randomly distributed across habitats and host species. First, using a GLMM (with site and parasite lineage as random factors), we showed that the probability of *Plasmodium* infection for a given individual varied between the lowland and the two other habitat types (fynbos: $t = -2.7$; $P = 0.005$; highland: $t = -3.0$; $P = 0.002$). Second, a randomization model indicated that seven *Plasmodium* lineages were preferentially associated with some host species in the lowland rainforest

habitat (i.e. the number of host species infected by these parasite lineages was significantly lower than expected with random association between parasites and individual birds, see details in Appendix S1, part B). No such association was found for the highland forest and fynbos habitats.

Host specificity index

A first comparison between habitats revealed that local S_{TD} indices were variable across habitats (ANOVA, $F_{2,84} = 27.4$, $P < 10^{-4}$). *Post hoc* tests indicated that this global effect was because of differences between the lowland rainforest and the two other habitat types ($P < 10^{-4}$ for both pair comparisons). Similar analysis with global S_{TD} showed that there was also a global difference between habitats (ANOVA, $F_{2,84} = 7.7$, $P = 0.0008$), but only the lowland and highland habitats differed from each other ($P = 0.005$). To check whether these differences were due only to the observed differences in parasite prevalence between habitats, we ran a linear mixed-effects models model with site and parasite lineage as random factors. The analysis indicated that the lowland still differed from the two other habitat types with respect to local (fynbos: $t = 1.9$; $P = 0.05$; highland: $t = 4.2$; $P < 10^{-4}$) and global (fynbos: $t = 2.7$; $P = 0.006$; highland: $t = 4.8$; $P < 10^{-4}$) S_{TD} , even when controlling for lineage.

We also found that the global host specificity index was negatively affected by the geographical range of the hosts ($F_{1,34} = 9.22$; $P = 0.0045$). Parasites with a high host specificity index (i.e. generalist parasites) were found in hosts with a limited geographical range. In contrast, hosts with large geographical ranges tended to be infected by parasites with a low host specificity index (i.e. specialist parasites).

Evolutionary relationships

The phylogenetic relationship from the consensus tree showing the 34 *Plasmodium* lineages revealed groups of lineages with different host and habitat specificities that we designated as clusters A, B and C (Fig. 1). Cluster A represents habitat generalist *Plasmodium* lineages showing a high host specificity index. Cluster B represents specialist lineages found only in four species of sunbirds (Nectariniidae: *Cyanomitra olivacea*, *C. cyanolaima*, *C. oritis* and *Hedydipna collaris*). The cluster C represents *Plasmodium* lineages primarily from lowland forests that have a low host specificity index. S_{TD} values from cluster A were significantly different from cluster B (Kruskal–Wallis test, $\chi^2 = 4.82$; $P = 0.028$), as well as from the cluster C (Kruskal–Wallis test, $\chi^2 = 8.23$;

$P = 0.0041$), but the values from cluster B and C did not significantly differ from one another.

Ancestral reconstruction analysis and likelihood ratio tests revealed a significant difference between the transition rates between generalist and specialist states (Equal rates $L = -21.810$, Unequal rates $L = -16.358$), with the rate at which generalists become specialists nearly four times (70.41) that of which specialists become generalists (17.63).

Discussion

It has been established that the degree of specialization of parasites lies along a continuum; on one end, extreme specialists may be restricted to a single host species, and on the other, generalists are capable of parasitizing a diverse set of avian hosts. However, there is little information about factors that can contribute to the evolution of host specificity. With ever increasing evidence for rapid global change, it is especially pertinent to establish how variation in environmental conditions can be linked to parasite strategies. Here, we take advantage of an unprecedented compilation of blood samples and field locations to compare diversity of malaria parasites across wide regions in Africa. In addition, to our knowledge, this is the first attempt to investigate avian malaria parasites and quantify a specificity index of host–parasite communities in relation to habitat characteristics. Using these host specificity data and our reconstructed phylogeny of these parasites, this study supports the hypotheses that (i) the diversity of parasites and prevalence differ among host communities and habitats in Africa, (ii) specialist parasites evolve from generalists more often than generalists evolve from specialists and (iii) the degree of host specialization is associated to habitat type and host geographical range.

We found that the prevalence and diversity of parasites varied considerably among three environmentally distinct habitats, and that host specificity indices of parasites differed significantly according to habitat. Results indicate that habitat type limits the geographical range of the host species, which in turn influences parasite specialization. The data revealed that generalist parasite lineages tend to infect bird communities with restricted geographical ranges as compared to those communities with larger ranges. Conversely, bird species with more extensive geographical ranges tend to be infected mostly with specialist parasites. Indeed, a parasite can specialize on a single host when the host occurs across an extensive environmental range, but would be at risk of rapid extinction in specializing on host species with limited geographical ranges. Only generalist parasites, with the ability to infect multiple species, would likely

infect these birds and persist. It is worth noting that the host specificity index is based not only on our data but also on data available from large databases that involve a significant number of screened hosts, to obtain the most reliable index possible. Although we cannot extend our conclusions to all lowland, highland or fynbos habitats in Africa, we hypothesize that the trends observed represent an advance in our understanding of mechanisms involved in parasite strategy evolution, and they further provide testable predictions for areas outside our study sites.

Random forest analysis used to classify the sites indicates that the three habitats clearly differ in terms of climatic conditions. Temperature seasonality (BIO04) as well as the minimum temperature of the coldest month (BIO06) distinguishes each of the three habitat types. Temperature variables can impact both hosts and vectors and consequently the abundance and diversity of parasites (Paaijmans *et al.* 2010). This is in accordance with several studies of human-associated malaria parasites and their vectors in the highlands of East Africa and elsewhere. Both the survival of vector populations and the development time of the parasite vary with diurnal temperature, and in field studies, temperature variation has been related to the abundance of vector populations (Koenraadt *et al.* 2006; Minakawa *et al.* 2006; Afrane *et al.* 2008; Paaijmans *et al.* 2009, 2010; Chaves & Koenraadt 2010; LaPointe *et al.* 2010). This type of climate variability (short-term fluctuations around the mean climate state) would, therefore, play an important role in host specialization of malaria parasites. For instance, altitude is inversely correlated to temperature. In the Usambara Mountains (Tanzania), the difference between the lowest village on the warm plains at 300 m and the coldest village at 1700 m is 9 °C (Bodker *et al.* 2003). Fewer vector species are expected in colder habitats, and these habitats may be likely to harbour more generalist parasites. In this study, however, one limitation is that we lack information on the vector ecology. The parasite–vector interactions and specificity are difficult to predict, as the mosquito vectors could be impacted in different ways by environmental conditions. To date, two studies have been carried out in the lowland forests of Cameroon, revealing that species of four genera of mosquitoes, *Aedes*, *Coquillettidia*, *Culex* and *Mansonia*, are potential vectors of avian malaria (Njabo *et al.* 2009, 2010). However, very little is known about the vectors transmitting avian malaria in Tanzania or South Africa. Collecting vectors and identifying the host specificity of their malaria parasites in the highland forest and the fynbos would be an important step in assessing the strategies of generalist parasites in these habitats.

In our phylogenetic analyses of *Plasmodium* lineages, we found clusters with different host and habitat specificities. Implementing ancestral reconstruction methodologies, we found that specialized lineages tend to be phylogenetically derived. Similar results have been seen in other parasitic systems, such as schistosomes (Snyder & Loker 2000). Our results support the evolutionary directionality of malaria parasites from generalist towards specialist, as the transition from generalist to specialist occurs at a significantly faster rate than the transition from specialist to generalist. In addition to this evolutionary transition, ecological factors drive host specialization. Indeed, it is becoming increasingly clear that the disturbance of habitat leads to homogenization of biodiversity (Clavel *et al.* 2010; Freedman *et al.* 2010; Hewitt *et al.* 2010; Laliberte & Tylianakis 2010) and will also lead to a loss of diversity in birds with restricted ranges. For instance, the mountains in Tanzania exhibit a high diversity of endemic birds, which are extremely sensitive to disturbance (Hall *et al.* 2009; Giam *et al.* 2010). In addition, we expect that some generalist vertebrate hosts and vectors will extend their habitat ranges, because of habitat disturbance (Ogden *et al.* 2008; Marini *et al.* 2009; Gonzalez *et al.* 2010), and carry with them novel lineages of parasites. This increased range sizes of parasites could be associated with increased parasite virulence and have serious impacts on host health (Garamszegi 2006). Our findings have conservation implications and are important in understanding the consequences of environmental change for malaria parasites. Ultimately, to fully test the hypotheses presented here, it will be important to assess the actual fitness effects of generalist versus specialist parasites on their hosts through experimental infections. The resulting information would be invaluable in assessing effects of global changes on the virulence of malaria parasites.

Acknowledgements

We thank the Governments of the Republic of Cameroon, Tanzania (The Tanzanian Commission for Science and Technology and Tanzanian Wildlife Research Institute), Malawi (Museums of Malawi), Mozambique (National Museum Maputo) and South Africa (CapeNature, Northern Cape Conservation) for permission to conduct field research. We thank Anthony Chasar, Kevin Njabo, Dennis Anye Ndeh, Jerome Fuchs, Graeme Oatley, Mhairi McFarlane, Martim Melo, Claire Spottiswoode, Penn Lloyd, Hanneline Smit, Ângela Ribeiro, Peter Ryan, Potiphar Kaliba, Jacob Kuire, Jan Bolding Kristensen, Louis Hansen, Jay McEntee and Jon Fjelds  for their help in the field, and Raul Sedano and Karine Princ  for their help with statistical analyses. We are also grateful to Fran ois Balloux and the anonymous reviewers for helpful comments on the manuscript. This research was supported by grants

from the National Geographic Society, National Environmental Research Council and the National Science Foundation DEB-9726425 and IRCEB9977072 and joint NSF-NIH (USA) Ecology of Infectious Diseases Program award EF-0430146 to TBS and RNMS. The work was also partially supported by the award NIH Grant 5SC2AI089120 to RNMS, as well as the University of California at Berkeley's committee on research, and the Department of Science and Technology and National Research Foundation of South Africa.

References

- Afrane YA, Little TJ, Lawson BW, Githeko AK, Yan G (2008) Deforestation and vectorial capacity of *Anopheles gambiae* Giles mosquitoes in malaria transmission, Kenya. *Emerging Infectious Diseases*, **14**, 1533–1538.
- Beadell JS, Covas R, Gebhard C *et al.* (2009) Host associations and evolutionary relationships of avian blood parasites from West Africa. *International Journal of Parasitology*, **39**, 257–266.
- Bensch S, Åkesson A (2003) Temporal and spatial variation of hematozoans in Scandinavian willow warblers. *Journal of Parasitology*, **89**, 388–391.
- Bensch S, Hellgren O, Perez-Tris J (2009) MalAvi: a public database of malaria parasites and related haemosporidians in avian hosts based on mitochondrial cytochrome b lineages. *Molecular Ecology Resources*, **9**, 1353–1358.
- Berg M, Kiers ET, Driessen G *et al.* (2010) Adapt or disperse: understanding species persistence in a changing world. *Global Change Biology*, **16**, 587–598.
- Bodker R, Akida J, Shayo D *et al.* (2003) Relationship between altitude and intensity of malaria transmission in the Usambara Mountains, Tanzania. *Journal Medical Entomology*, **40**, 706–717.
- Bonneaud C, Sepil I, Mil  B *et al.* (2009) The prevalence of avian Plasmodium is higher in undisturbed tropical forests of Cameroon. *Journal of Tropical Ecology*, **25**, 439–447.
- Both C, te Marvelde L (2007) Climate change and timing of avian breeding and migration throughout Europe. *Climate Research*, **35**, 93–105.
- Brooks DR, Hoberg EP (2008) How will global climate change affect parasite-host assemblages? *Trends of Parasitology*, **23**, 571–574.
- Chasar A, Loiseau C, Valki nas G, Iezhova T, Smith TB, Sehgal RNM (2009) Prevalence and diversity patterns of avian blood parasites in degraded African rainforest habitats. *Molecular Ecology*, **18**, 4121–4133.
- Chaves LF, Koenraad CJ (2010) Climate change and highland malaria: fresh air for a hot debate. *Quarter Review of Biology*, **85**, 27–55.
- Clavel J, Julliard R, Devictor V (2010) Worldwide decline of specialist species: towards a global functional homogenization? *Frontiers in Ecology and the Environment*. doi: 10.1890/080216.
- Colles A, Liow LH, Prinzing A (2009) Are specialists at risk under environmental change? Neocological, paleoecological and phylogenetic approaches. *Ecology Letters*, **12**, 849–863.
- Crawley MJ (2007) *The R Book*. John Wiley & Sons Ltd, Chichester.
- De Klerk HM, Crowe TM, Fjelds  J, Burgess ND (2002) Biogeographical patterns of endemic terrestrial Afrotropical birds. *Diversity and Distributions*, **8**, 147–162.

- Devictor V, Julliard R, Clavel J, Jiguet F, Lee A, Couvet D (2008) Functional biotic homogenization of bird communities in disturbed landscapes. *Global Ecology and Biogeography*, **17**, 252–261.
- Ekroos J, Heliola J, Kuussaari M (2010) Homogenization of lepidopteran communities in intensively cultivated agricultural landscapes. *Journal Applied of Ecology*, **47**, 459–467.
- Fairbanks DHK, Hughes CJ, Turpie JK (2004) Potential impact of viticulture expansion on habitat types in the Cape Floristic Region, South Africa. *Biodiversity and Conservation*, **13**, 1075–1100.
- Fisher DO, Blomberg SP, Owens IPF (2003) Extrinsic versus intrinsic factors in the decline and extinction of Australian marsupials. *Proceeding of the Royal Society of London B*, **270**, 1801–1808.
- Foufopoulos J, Ives AR (1999) Reptile extinctions on land-bridge islands: life-history attributes and vulnerability to extinction. *American Naturalist*, **153**, 1–25.
- Freedman AH, Buermann W, Mitchard ETA, DeFries RS, Smith TB (2010) Human impacts flatten rainforest-savanna gradient and reduce adaptive diversity in a rainforest bird. *PLoS ONE*, **5**, e13088.
- Garamszegi LZ (2006) The evolution of virulence and host specialization in malaria parasites of primates. *Ecology Letters*, **9**, 933–940.
- Garamszegi LZ (2011) Climate change increases the risk of malaria in birds. *Global Change Biology*, **17**, 1751–1759.
- Giam X, Bradshaw CJA, Tan HTW, Sodhi NS (2010) Future habitat loss and the conservation of plant biodiversity. *Biological Conservation*, **143**, 1594–1602.
- Gomez JM, Verdu M, Perfectti F (2010) Ecological interactions are evolutionarily conserved across the entire tree of life. *Nature*, **465**, 918–921.
- Gonzalez C, Wang O, Strutz SE, Gonzalez-Salazar C, Sanchez-Cordero V, Sarkar S (2010) Climate change and risk of Leishmaniasis in North America: predictions from ecological niche models of vector and reservoir species. *Plos Neglected Tropical Disease*, **4**, e585.
- Gordo O (2007) Why are bird migration dates shifting? A review of weather and climate effects on avian migratory phenology. *Climate Research*, **35**, 37–58.
- Hall J, Burgess ND, Lovett J, Mbilinyi B, Gereau RE (2009) Conservation implications of deforestation across an elevational gradient in the Eastern Arc Mountains, Tanzania. *Biological Conservation*, **142**, 2510–2521.
- Hansen MC, DeFries RS, Townshend JRG, Sohlberg R, Dimiceli C, Carroll M (2002) Towards an operational MODIS continuous field of percent tree cover algorithm: examples using AVHRR and MODIS data. *Remote Sensing of Environment*, **83**, 303–319.
- Harcourt AH, Copeton SA, Parks SA (2002) Rarity, specialization and extinction risk in primates. *Journal of Biogeography*, **29**, 445–456.
- Hellgren O, Waldenström J, Perez-Tris J *et al.* (2007) Detecting shifts of transmission areas in avian blood parasites - a phylogenetic approach. *Molecular Ecology*, **16**, 1281–1290.
- Hellgren O, Perez-Tris J, Bensch S (2009) A jack-of-all-trades and still a master of some: prevalence and host range in avian malaria and related blood parasites. *Ecology*, **90**, 2840–2849.
- Hewitt J, Thrush S, Lohrer A, Townsend M (2010) A latent threat to biodiversity: consequences of small-scale heterogeneity loss. *Biodiversity and Conservation*, **19**, 1315–1323.
- Hijmans RJ, Cameron SE, Parra JL, Jones PG, Jarvis A (2005) Very high resolution interpolated climate surfaces for global land areas. *International Journal of Climatology*, **25**, 1965–1978.
- Hitch AT, Leberg PL (2007) Breeding distributions of north American bird species moving north as a result of climate change. *Conservation Biology*, **21**, 534–539.
- Huelsenbeck JP, Ronquist F (2001) MRBAYES: Bayesian inference of phylogenetic trees. *Bioinformatics*, **17**, 754–755.
- Huete AR, Didan K, Shimabukuro YE *et al.* (2006) Amazon rainforests green-up with sunlight in dry season. *Geophysical Research Letters*, **33**, L06405, doi:10.1029/2005GL025583.
- Hughes JB, Daily GC, Ehrlich PR (2000) Conservation of insect diversity: a habitat approach. *Conservation Biology*, **14**, 1788–1797.
- Ishtiaq F, Clegg SM, Phillimore AB, Black R, Owens IPF, Sheldon BC (2010) Biogeographical patterns of blood parasite lineage diversity in avian hosts from southern Melanesian islands. *Journal of Biogeography*, **37**, 120–132.
- Johnson KP, Malenke JR, Clayton DH (2009) Competition promotes the evolution of host generalists in obligate parasites. *Proceeding of the Royal Society of London B*, **276**, 3921–3926.
- Julliard R, Jiguet F, Couvet D (2004) Common birds facing global changes: what makes a species at risk? *Global Change Biology*, **10**, 148–154.
- Kelley ST, Farrell BD (1998) Is specialization a dead end? The phylogeny of host use in Dendroctonus bark beetles (Scolytidae). *Evolution*, **52**, 1731–1743.
- Kirkpatrick CE, Smith TB (1988) Blood parasites of birds in Cameroon. *Journal of Parasitology*, **74**, 1009–1013.
- Knowles SCL, Wood MJ, Alves R, Wilkin TA, Bensch S, Sheldon BC (2010) Molecular epidemiology of malaria prevalence and parasitaemia in a wild bird population. *Molecular Ecology*, doi: 10.1111/j.1365-294X.2010.04909.
- Koenraadt CJM, Paaijans KP, Schneider P, Githeko AK, Takken W (2006) Low larval vector survival explains unstable malaria in the western Kenya highlands. *Tropical Medicine and International Health*, **11**, 1195–1205.
- Kotze DJ, O'Hara RB (2003) Species decline – but why? Explanations of carabid beetle (Coleoptera, Carabidae) declines in Europe. *Oecologia*, **135**, 138–148.
- Lafferty KD (2009) The ecology of climate change and infectious diseases. *Ecology*, **90**, 888–900.
- Laliberte E, Tylianakis JM (2010) Deforestation homogenizes tropical parasitoid-host networks. *Ecology*, **91**, 1740–1747.
- LaPointe DA, Goff ML, Atkinson CT (2010) Thermal constraints to the sporogonic development and altitudinal distribution of avian malaria *Plasmodium relictum* in Hawai'i. *Journal of Parasitology*, **96**, 318–324.
- Liaw A, Wiener M (2002) Classification and regression by randomForest. *R News*, **2**, 18–22.
- Loiseau C, Iezhova T, Valkiunas G *et al.* (2010) Spatial variation of haemosporidian parasite infection in African rainforest bird species. *Journal of Parasitology*, **96**, 21–29.
- Marini MA, Barbet-Massin M, Lopes LE, Jiguet F (2009) Predicted climate-driven bird distribution changes and

- forecasted conservation conflicts in a neotropical savanna. *Conservation Biology*, **23**, 1558–1567.
- Massad E, Coutinho FAB, Lopez LF, da Silva DR (2011) Modeling the impact of global warming on vector-borne infections. *Physics of Life Reviews*, **8**, 169–199.
- McKinney ML (1997) Extinction vulnerability and selectivity: combining ecological and paleontological views. *Annual Review of Ecology, Evolution and Systematics*, **28**, 495–516.
- Merilä J, Bjorklund M, Bennett GF (1995) Geographic and individual variation in haematozoan infections in the greenfinch, *Carduelis chloris*. *Canadian Journal of Zoology*, **73**, 798–1804.
- Midgley GF, Hannah L, Millar D, Rutherford MC, Powrie LW (2002) Assessing the vulnerability of species richness to anthropogenic climate change in a biodiversity hotspot. *Global Ecology and Biogeography*, **11**, 445–451.
- Minakawa N, Omukunda E, Zhou GF, Githeko A, Yan GY (2006) Malaria vector productivity in relation to the highland environment in Kenya. *American Journal of Tropical Medicine and Hygiene*, **75**, 448–453.
- Mostowj R, Engelstaedter J (2011) The impact of environmental change on host-parasite coevolution dynamics. *Proceeding Royal Society London B*, **278**, 2283–2292.
- Munday PL (2004) Habitat loss, resource specialization, and extinction on coral reefs. *Global Change Biology*, **10**, 1642–1647.
- Njabo K, Cornel AJ, Sehgal RNM *et al.* (2009) Coquillettidia (Culicidae, Diptera) mosquitoes are natural vectors of avian malaria in Africa. *Malaria Journal*, **8**, 193.
- Njabo K, Cornel AJ, Bonneaud C *et al.* (2010) Nonspecific patterns of vector, host and avian malaria parasite associations in a central African rainforest. *Molecular Ecology*, **20**, 1049–1061.
- Nosil P (2002) Transition rates between specialization and generalization in phytophagous insects. *Evolution*, **56**, 1701–1706.
- Nylander JAA (2004) *MrModeltest v2*. Program distributed by the author. Evolutionary Biology Centre, Uppsala University, Uppsala, Sweden.
- Ogden NH, St-Onge L, Barker IK *et al.* (2008) Risk maps for range expansion of the Lyme disease vector, *Ixodes scapularis*, in Canada now and with climate change. *International Journal of Health Geographics*, **7**, 24.
- Owens IPF, Bennett PM (2000) Ecological basis of extinction in birds: habitat loss versus human persecution and introduced predators. *Proceedings of the National Academy of Science*, **97**, 12144–12148.
- Paaijmans KP, Read AF, Thomas MB (2009) Understanding the link between malaria risk and climate. *Proceedings of the National Academy of Science*, **106**, 13844–13849.
- Paaijmans KP, Blanford S, Bell AS, Blanford J, Read AF, Thomas MB (2010) Influence of climate on malaria transmission depends on daily temperature variation. *Proceedings of the National Academy of Science*, **107**, 15135–15139.
- Pagel M (1999) The maximum likelihood approach to reconstructing ancestral character states of discrete characters on phylogenies. *Systematic Biology*, **48**, 612–622.
- Poulin R, Mouillot D (2003) Parasite specialization from a phylogenetic perspective: a new index of host specificity. *Parasitology*, **126**, 473–480.
- Poulin R, Krasnov BR, Shenbrot GI, Mouillot D, Khoklova S (2006) Evolution of host specificity in fleas: is it directional and irreversible? *International Journal of Parasitology*, **36**, 185–191.
- Poulin R, Krasnov BR, Mouillot D (2011) Host specificity in phylogenetic and geographic space. *Trends in Parasitology*, **27**, 355–361.
- Pounds JA, Fogden MP, Campbell JH (1999) Biological response to climate change on a tropical mountain. *Nature*, **398**, 611–615.
- R Development Core Team (2009) *R: A Language and Environment for Statistical Computing*. V. 2.7.2. [http://www.R-project.org].
- R Development Core Team: R (2004) *A Language and Environment for Statistical Computing*. R Foundation for Statistical Computing 2004, Vienna, Austria.
- Ricklefs RE, Fallon SM (2002) Diversification and host switching in avian malaria parasites. *Proceeding Royal Society London B*, **269**, 885–892.
- Ricklefs RE, Fallon SM, Bermingham E (2004) Evolutionary relationships, cospeciation, and host switching in avian malaria parasites. *Systematic Biology*, **53**, 111–119.
- SAS (1999) *SAS User's Guide*. SAS Institute Inc., Cary, NC, USA.
- Sehgal RNM (2010) Deforestation and avian infectious diseases. *Journal of Experimental Biology*, **213**, 955–960.
- Sehgal RNM, Lovette IJ (2003) Molecular evolution of three avian neurotrophin genes: Implications for proregion functional constraints. *Journal of Molecular Evolution*, **57**, 335–342.
- Sehgal RNM, Jones HI, Smith TB (2005) Blood parasites of some West African rainforest birds. *The Journal of Veterinary Medical Science*, **67**, 295–301.
- Sehgal RNM, Buermann W, Harrigan RJ *et al.* (2011) Spatially explicit predictions of blood parasites in a widely distributed African rainforest bird. *Proceeding of the Royal Society London B*, **278**, 1025–1033.
- Snyder SD, Loker ES (2000) Evolutionary relationships among the Schistosomatidae (Platyhelminthes: Digenea) and an Asian origin for *Schistosoma*. *Journal of Parasitology*, **86**, 283–288.
- Svensson LME, Ricklefs RE (2009) Low diversity and high intra-island variation in prevalence of avian Haemoproteus parasites on Barbados, Lesser Antilles. *Parasitology*, **136**, 1121–1131.
- Swofford DL (2003) *PAUP*: Phylogenetic Analysis Using Parsimony (*and Other Methods)*. Version 4. Sinauer Associates, Sunderland, Massachusetts.
- Tabachnick WJ (2010) Challenges in predicting climate and environmental effects on vector-borne disease epizootics in a changing world. *Journal of Experimental Biology*, **213**, 946–954.
- Thomas F, Brown SP, Sukhdeo M, Renaud F (2002) Understanding parasite strategies: a state-dependent approach? *Trends of Parasitology*, **18**, 387–390.
- Tonnang HEZ, Kangalawe RYM, Yanda PZ. 2010. Predicting and mapping malaria under climate change scenarios: the potential redistribution of malaria vectors in Africa. *Malaria Journal*, **9**, 111.
- Valkiūnas G (2005) *Avian Malaria Parasites and Other Haemosporidia*. CRC Press, Boca Raton, Florida, 946 p.

- Valkiūnas G, Iezhova TA, Loiseau C, Chasar A, Smith TB, Sehgal RN (2008a) New species of haemosporidian parasites (Haemosporida) from African rainforest birds, with remarks on their classification. *Parasitology Research*, **103**, 1213–1228.
- Valkiūnas G, Iezhova TA, Krizanauskiene A, Palinauskas V, Sehgal RN, Bensch S (2008b) A comparative analysis of microscopy and PCR-based detection methods for blood parasites. *Journal of Parasitology*, **94**, 1395–1401.
- Waldenström J, Bensch S, Kiboi S, Hasselquist D, Ottoson U (2002) Cross-species infection of blood parasites between resident and migratory songbirds in Africa. *Molecular Ecology*, **11**, 1545–1554.
- Waldenström J, Bensch S, Hasselquist D, Ostman O (2004) A new nested polymerase chain reaction method very efficient in detecting *Plasmodium* and *Haemoproteus* infections from avian blood. *Journal of Parasitology*, **90**, 191–194.
- Wood MJ, Cosgrove CL, Wilkin TA, Knowles SCL, Day KP, Sheldon BC (2007) Within-population variation in prevalence and lineage distribution of avian malaria in blue tits, *Cyanistes caeruleus*. *Molecular Ecology*, **16**, 3263–3273.
- World Health Organization (2009) Malaria. WHO Fact Sheet No. 94.
- Zell R (2004) Global climate change and the emergence/re-emergence of infectious diseases. *International Journal of Medical Microbiology*, **293**, 16–26.

C.L. is interested in ecology of avian parasitic diseases and the anthropogenic impacts on bird communities. R.H.'s main research interests include the evolutionary biology and ecology of species complexes. A.R. has broad interests in population genetics and conservation biology. R.B.'s main research interests include biogeography and speciation processes in birds, with a particular emphasis on African taxa. H.T. is interested in the evolutionary processes generating and maintaining biodiversity. T.S. is interested in evolutionary genetics, speciation and the conservation of tropical vertebrates. R.S.'s broad interests are the ecology and evolution of blood-borne pathogens of birds.

Data accessibility

New DNA sequences: Genbank accessions HQ022817–HQ022822.

Climate data for each site deposited in the DRYAD repository entry: doi:10.5061/dryad.h12kh08n.

Supporting information

Additional supporting information may be found in the online version of this article.

Appendix S1 Bioclimatic and habitat variables description and methods.

Fig. S1 Location of sampling sites in each of the three habitats; lowlands, highlands, and fynbos.

Fig. S2 Spatial autocorrelation in *Plasmodium* spp. prevalence in each of the three habitats; lowlands, highlands, and fynbos.

Table S1 Coordinates and altitude at each sites (*) represents sites where *Plasmodium* infection was found.

Table S2 Number of individual screened (NTotal) and number of infected individuals (Ninf) per host species.

Table S3 Number of individual screened (NTotal) and number of infected individuals (Ninf) per host species.

Table S4 Number of individual screened (NTotal) and number of infected individuals (Ninf) per host species.

Table S5 Host specificity indices are given for each *Plasmodium* lineage found in each habitat.

Table S6 Distances (Km²) between each site in the lowland West Africa habitat.

Table S7 Distances (Km²) between each site in the highland East Africa habitat.

Table S8 Distances (Km²) between each site in the fynbos in South Africa.

Please note: Wiley-Blackwell are not responsible for the content or functionality of any supporting information supplied by the authors. Any queries (other than missing material) should be directed to the corresponding author for the article.