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Host and habitat specialization of avian malaria in Africa

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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 vs. 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

Plasmodium; avian malaria; habitat specificity; specialist; generalist

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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: Hugues *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; Ekroos *et al.* 2010; Clavel *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 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 due to 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.* 2010; 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).

Due to 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 versus 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 (DeKlerk *et al.* 2002). In the near future, the communities of birds in these habitats will likely suffer declines due to 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 since they are temperature sensitive (Tonnang *et al.* 2010; Paaijmans *et al.* 2009) and the community of birds, and should harbor 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, 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 Electronic Supplemental Information Table S1 and distances between sites in Tables S6–S8; see also map in Fig. S1). 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 (NDVI; Huete *et al.* 2006), the percentage of tree cover (Hansen *et al.* 2002) and elevation data from the Shuttle Radar Topography Mission (SRTM) (see Electronic Supplemental Information 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 8 bird species from 5 families among the 9 West African lowland forest sites ($N = 575$; Table S2), 11 bird species from 5 families at 17 sites in East African highland

forests ($N = 406$; Table S3) and 6 bird species from four families at 10 sites in South Africa in the fynbos ($N = 383$; Table S4). 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, California). Successful DNA extraction was verified with primers that amplify the brain-derived neurotrophic factor (BDNF) (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 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, Ohio). 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 are not detected by PCR; however Valkiūnas *et al.* (2008b) demonstrated that both of 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 (Electronic Supplemental Information, part B). In addition, in order 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 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 to these expectations (details presented in Electronic Supplemental Information, 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 get 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; Hellgren *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, we combined all available data to estimate a global host specificity index. Host specificity indices are given in Table S5.

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 LMM, 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). LMM was used to assess whether particular parasite lineages show differences in S_{TD} in the different habitats. GLM, LMM 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). 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.mbioekol.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 (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 (ML) in PAUP* 4.0 (Swofford 2003). The support for the individual branches was estimated using ML bootstrap analyses with 100 replicates. The topology of both the Bayesian and the ML trees were 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; 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 (Electronic Supplemental Information, part D).

Results

Parasite prevalence, diversity and distribution

In total, from 25 bird species (1,364 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).

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 Electronic Supplemental Information, 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 due to 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 ($P = 0.005$). In order to check whether these differences were due only to the observed differences in parasite prevalence between habitats (see above), we ran a LMM 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. cyanolaema*, *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 data available from large databases that involve a significant number of screened hosts, in order 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) distinguish 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; Chaves *et al.* 2010; La Pointe *et al.* 2010; Paaijmans *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 1,700 m is 9°C (Bodker *et al.* 2003). Fewer vector species are expected in colder habitats, and these habitats may be likely to harbor 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 since 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; Njabo *et al.* 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 (Laliberte & Tylianakis 2010; Hewitt *et al.* 2010; Clavel *et al.* 2010; Freedman *et al.* 2010) and will also lead to a loss of diversity of 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.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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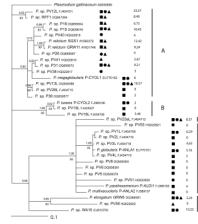


Figure 1.

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 ST_D index is given for each lineage. The bars indicate three groups of parasites (A: host generalist; B: host specialist; C: host specialist).

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	Ngoia	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
Fynbos	Mokoko	63	19	9	30.2
	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
	Kilimanjaro	2	1	1	50.0
	WestUsambara	43	1	1	2.3
	EastUsambara	19	4	2	21.1
	Nguru	56	13	5	23.2
Highland	Rubebo	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