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Prevalence and beta diversity in avian malaria communities: host species is a better predictor than geography

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Summary

1. Patterns of diversity and turnover in macroorganism communities can often be predicted from differences in habitat, phylogenetic relationships among species and the geographical scale of comparisons. In this study, we asked whether these factors also predict diversity and turnover in parasite communities.

2. We studied communities of avian malaria in two sympatric, ecologically similar, congeneric host species at three different sites. We asked whether parasite prevalence and community structure varied with host population, host phylogeography or geographical distance.

3. We used PCR to screen birds for infections and then used Bayesian methods to determine phylogenetic relationships among malaria strains. Metrics of both community and phylogenetic beta diversity were used to examine patterns of malaria strain turnover between host populations, and partial Mantel tests were used determine the correlation between malaria beta diversity and geographical distance. Finally, we developed microsatellite markers to describe the genetic structure of host populations and assess the relationship between host phylogeography and parasite beta diversity.

4. We found that different genera of malaria parasites infect the two hosts at different rates. Within hosts, parasite communities in one population were phylogenetically clustered, but there was otherwise no correlation between metrics of parasite beta diversity and geographical or genetic distance between host populations. Patterns of parasite turnover among host populations are consistent with malaria transmission occurring in the winter rather than on the breeding grounds.

5. Our results indicate greater turnover in parasite communities between different hosts than between different study sites. Differences in host species, as well as transmission location and vector ecology, seem to be more important in structuring malaria communities than the distance–decay relationships frequently found in macroorganisms. Determining the factors affecting parasite community diversity and turnover has wide-ranging implications for understanding the selective pressures shaping host ecology and ecosystem structure. This study shows that metrics of community and phylogenetic beta diversity can be useful tools for disentangling the ecological and evolutionary processes that underlie geographical variation in parasite communities.

Key-words: avian malaria, beta diversity, *Phylloscopus humei*, *Phylloscopus trochiloides*, phylogenetic beta diversity

Introduction

Decades of research on the factors structuring ecological communities have revealed broad-scale patterns of biodiversity turnover in macroorganisms: at regional scales, the

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similarity of biological communities frequently declines with increasing distance between communities ('distance–decay', Nekola & White 1999; Morlon *et al*. 2008; Pavoine & Bonsall 2011) and with changes in habitat types (e.g. via ecological replacement along mountainsides, Price & Richman 1992; with latitude, Soininen, McDonald & Hillebrand 2007). Recent studies have examined whether the same patterns of turnover also occur in micro-organisms, with mixed results. Communities of microbes can exhibit different patterns of turnover across habitat types compared to macroorganisms (e.g. elevational gradients, Bryant *et al*. 2008; precipitation gradients, Bachar *et al*. 2010), but there is also evidence for classic patterns of declining similarity with distance (Green *et al*. 2004; Martiny *et al*. 2006; Soininen, McDonald & Hillebrand 2007). Despite the important role of micro-organisms in shaping ecological communities, data on patterns of distribution and turnover remain sparse (Green *et al*. 2004).

Parasites are a ubiquitous selective pressure in the landscape; it is often estimated that every free-living organism is host to at least one parasite (Poulin 1996). Parasites consequently can influence nearly all aspects of host life history, from survival to mating strategies to habitat use to immune systems (e.g. Clayton *et al*. 1999; Marzal *et al*. 2005; De Coster *et al*. 2010). Parasite communities vary widely both temporally and spatially, resulting in a variable selective landscape that can lead to local adaptation and co-evolution between parasites and hosts (e.g. Bensch & Åkesson 2003; Knudsen *et al*. 2010). Although variation in the parasite community thus has the potential to shape the broader ecosystem, the factors influencing variation and turnover in parasites are poorly understood. Some studies of ectoparasites and gut endoparasites have found distance–decay relationships related to geographical distance among host populations (Poulin 2003; Oliva & González 2005; Whiteman, Kimball & Parker 2007), but others on blood-borne parasites have found no influence of host geography on parasite communities, and instead shown important effects of host and vector ecology (e.g. Ishtiaq *et al*. 2009; Svensson-Coelho & Ricklefs 2011).

Traditional metrics of community diversity and turnover ('alpha' and 'beta' diversity, respectively, *sensu* Whittaker 1960) use numbers and/or frequencies of shared and unique species to describe spatial variation in community composition (reviewed in Tuomisto 2010). These metrics have recently been extended to analyses of phylogenetic beta diversity that account for relatedness among species when describing patterns of abundance and/or distribution (Graham & Fine 2008). Measuring the phylogenetic distance between species within and among communities affords additional insight into the ecological and evolutionary processes structuring those communities (Webb *et al*. 2002; Graham & Fine 2008). For example, large phylogenetic distances among species in a community (phylogenetic overdispersion) are often indicative of competition, because close relatives cannot occupy the same ecological niche. Conversely, phylogenetic clustering (small phylogenetic distances) may reflect ecological filtering, in which a species assembly is made up of related species (Webb *et al*. 2002; Graham & Fine 2008; Cavender-Bares *et al*. 2009). Between communities, the location of turnover (beta diversity) on the phylogenetic tree is also informative: for example, turnover near the base of the tree indicates large differences in the evolutionary history of different assemblages, whereas turnover at the tips indicates fine-scale differences between communities of related species. In parasites, phylogenetic clustering of parasite strains within a host species, or a strong correlation between parasite strains and different host populations, could be indicative of host specificity of those strains. Conversely, phylogenetic overdispersion of parasite strains could reflect competition among related strains within a host.

Here, we use measures of prevalence, beta diversity and phylogenetic beta diversity to ask whether turnover of avian malaria communities in two host species can be explained by geographical distance and/or genetic structure of host populations. Avian malaria is a well-studied haemosporidian parasite that infects birds world-wide (Greiner *et al*. 1975; Atkinson & van Riper 1991; Pérez-Tris *et al*. 2005) and has fitness consequences for hosts in the form of reduced reproductive success (Marzal *et al*. 2005) and survival (Martínez-de la Puente *et al*. 2010). Two widespread and morphologically distinct genera of malaria parasites infect birds: *Plasmodium* and *Haemoproteus*. *Plasmodium* is transmitted by mosquito vectors and tends to contain generalist strains, whereas the more common *Haemoproteus* is transmitted by midges and exhibits comparatively greater host specificity (Atkinson & van Riper 1991; Valkiūnas 2005; Valkiūnas *et al*. 2013).

We studied avian malaria prevalence, strain diversity and community structure in two congeneric host species, *Phylloscopus humei* (Hume's warbler) and *Phylloscopus trochiloides* (Greenish warbler), which occupy similar ecological niches (Price & Gross 2005). Both warblers breed sympatrically in the Palearctic and overwinter parapatrically in central India (Katti & Price 2003; Price & Gross 2005). We studied these species at three breeding sites along a 2400 km north–south latitudinal gradient in Asia. The sites vary substantially in forest structure and food abundance, but both warblers occupy approximately the same habitat at each site (Scordato 2012). We addressed four questions related to the influence of host biology on parasite community structure:

1. Do more geographically distant host populations have more different parasite communities?
2. Do more genetically divergent host populations have more divergent parasite communities, after accounting for geography?
3. Are malaria communities most similar between different host species at the same study site, as expected if parasite communities vary across different habitats and/or transmission occurs between species?
4. Are malaria communities more similar across multiple populations of the same host species than between dif-

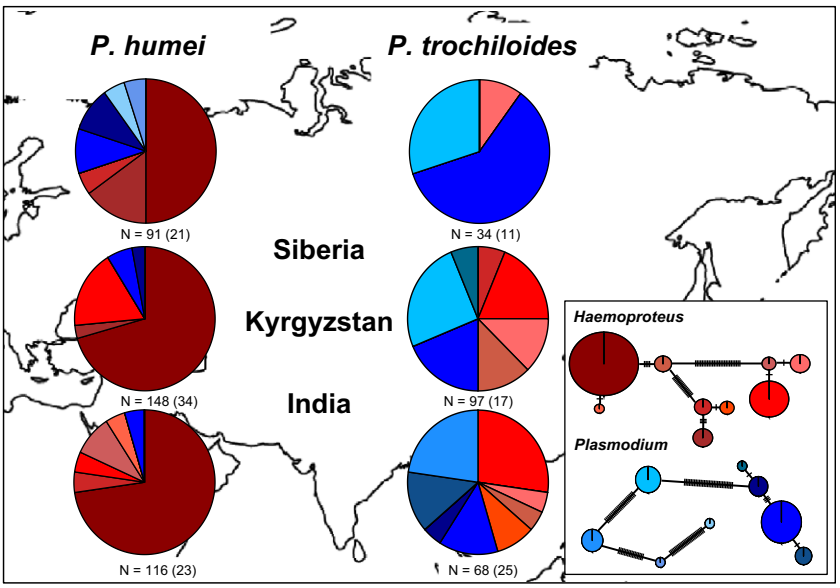


Fig. 1. Distribution of malaria strains in three populations of two host species. India and Siberia are approximately 2400 km apart, and with Kyrgyzstan equidistant between the two. *Haemoproteus* strains are shown in red shades and *Plasmodium* in blue shades. The size of each pie chart section corresponds to the proportion of infected individuals carrying a particular strain at each study site. Sample sizes are given for number of individuals screened for each species and site, with numbers of infected individuals in parentheses. *Phylloscopus humei*, at left, is infected primarily with *Haemoproteus*, although the proportion of *Plasmodium* infections increases with latitude. *Phylloscopus trochiloides*, at right, is infected primarily with *Plasmodium*, which occur at the highest frequency in Siberia. There is frequent host switching among strains, and some strains occur in both host species and across multiple sites. Inset: mtDNA haplotype networks for both genera of malaria parasites. Each dash shows one nucleotide difference, and size of circles indicates relative abundance of that strain.

ferent host species at the same site, as expected if parasites are host-specific and/or if transmission occurs on the wintering grounds?

Methods

We quantified prevalence and diversity of infections by different malaria genera within each host population. We then calculated three metrics of community and phylogenetic diversity for all pairwise comparisons between host populations to ask whether parasite communities were more different between host species, study sites or with different geographical or genetic distances. We chose metrics (Sørensen's dissimilarity index, β-NRI and β-NTI) that are easily interpretable and comparable across studies and allow us to ask whether observed patterns can be explained by phylogenetic relationships among malaria strains.

STUDY SITES AND DATA COLLECTION

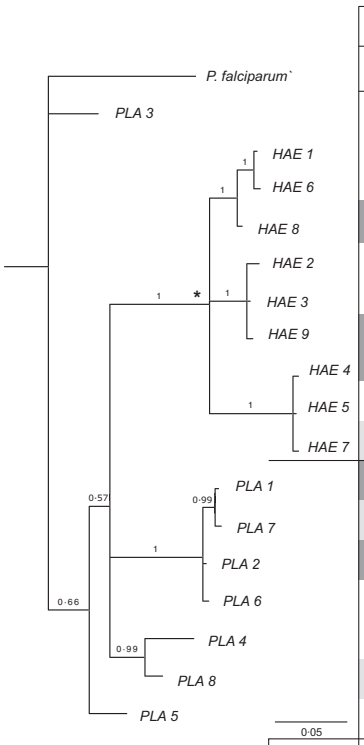
We collected blood samples from *P. trochiloides* (n = 199) and *P. humei* (n = 355) during the breeding season at four sites: Manali Sanctuary (elevation 3200 m, 77·176008°N, 32·238363°E) and Keylong Forest Preserve (3300 m, 32·50086°N, 76·97841°E), India (2007–2008); Ala Archa Park, Kyrgyzstan (2600 m, 42·54669°N, 74·48806°E, 2009); and Tigirek Nature Reserve, Siberia, Russia (2100 m, 51·123353°N, 83·037105°E, 2010). The sites in India are ~50 km apart, and samples have therefore been pooled in this analysis. The three populations lie along a 2400 km north–south transect, with Kyrgyzstan roughly intermediate between India and Siberia (Fig. 1). Male birds were captured in mist nets when they arrived on the breeding grounds in April and May, and females and some males were caught while feeding nestlings (June and July). A small blood sample was collected from the brachial vein of each bird and blotted on EDTA-treated filter paper. We collected blood samples from nestlings 10 days post-hatching.

GENETIC ANALYSIS

We extracted genomic DNA from blood samples using DNeasy Blood and Tissue Kits (Qiagen, Alameda, CA, USA). Samples from *P. humei* were typed at six microsatellite loci following the protocol described in Scordato, Bontrager and Price (2012). *P. trochiloides* were typed at 8 loci using microsatellite markers developed by B. Harr (see Appendix S1 in the Supporting Information). PCRs were run in multiplex using fluorescently labelled primers and genotyped at the University of Chicago Cancer Research Center. Alleles were scored in GeneMarker as described in Scordato, Bontrager and Price (2012). Phylogenetic trees for malaria strains were constructed in MrBayes v. 3.1.2. (Ronquist & Huelsenbeck 2003), and host genetic structure was analysed by calculating pairwise F_{ST} in Arlequin v. 3.5 (Excoffier, Laval & Schneider 2005) and assessing population clustering in STRUCTURE (Pritchard, Stephens & Donnelly 2000). See Appendix S1 for details of genetic analyses.

PREVALENCE ANALYSIS

We used generalized linear models (GLMs) to examine variation in infection prevalence across host species, sites and strain genus (*Haemoproteus* or *Plasmodium*) in adult birds. We constructed a maximal model containing all three explanatory variables and their interactions, with the presence or absence of infection as the



	P. trochiloides			*P. humei*		
	India	Kyrgyzstan	Siberia	India	Kyrgyzstan	Siberia
HAE 1						1
HAE 6				15	24	10
HAE 8				1		
HAE 2	1	2		1		
HAE 3					1	3
HAE 9		1		1		1
HAE 4	3			1		
HAE 5	5	3		1	6	
HAE 7				2		
PLA 1	1	2	1			
PLA 7	3	3	6	1	2	2
PLA 2	4					
PLA 6	1				1	2
PLA 4		1				
PLA 8			1			
PLA 5	5					
		4	3			
Infected	**23**	**16**	**11**	**23**	**34**	**19**
Total screened	**68**	**97**	**34**	**116**	**148**	**91**

Fig. 2. Left: Bayesian phylogeny of all unique malaria haplotypes with *P. falciparum* out-group. *P. falciparum* branch is shortened for illustrative purposes; all other branch lengths can be determined from the scale bar. *Haemoproteus* and *Plasmodium* fall into two reasonably well-supported clades. Star at the base of the *Haemoproteus* clade denotes node with more terminal taxa than expected based on null model of diversification. Right: number of individuals infected with each malaria haplotype in each host species and site. Black fill is strains unique to *P. humei*, light grey fill is strains unique to *P. trochiloides*, and dark grey fill denotes strains found in both host species. Total numbers of individuals screened and numbers of those infected for each host species at each site are shown at bottom.

response variable and a binomial error structure. We used backwards-stepwise model selection to drop non-significant terms from the model, starting with the three-way interaction and testing each possible two-way interaction. We also constructed models for each species separately, including the interaction between strain genus, sex and site in the maximal model and following the same stepwise model selection.

DIVERSITY ANALYSES

To quantify changes in the composition of parasite communities, we used both standard and phylogenetic metrics of diversity. In these analyses, each unique malaria haplotype, or strain, is treated as a separate 'species.' Since we found substantial variation in the prevalence of different malaria strains (Fig. 2), all our metrics are weighted by abundance, measured as the number of hosts infected with a particular haplotype. Each of these unique haplotypes is unlikely to be a true species. It has been suggested that haplotypes differing by <0·5% sequence divergence may be variants of the same strain (Bensch *et al.* 2004), and some haplotypes we identified differ by only 0·2% sequence divergence (Fig. 2). However, since species delineations in micro-organisms are difficult and controversial (Bensch *et al.* 2004; Ricklefs *et al.* 2011), and because many of the strains that differed by <0·5% divergence were nonetheless unique to different hosts and/or sites (e.g. strains HAE4, HAE5 and HAE7 in Fig. 2), we opted to treat each haplotype as a separate lineage or 'species.' This maximizes our ability to detect fine-scale patterns of host- or site-specific turnover in different malaria lineages, but means our analyses reflect turnover in genetic variants, not necessarily 'species.'

There are a variety of diversity indices available in the literature (reviewed in Webb *et al.* 2002; Webb, Ackerly & Kembel 2008; Swenson 2011), but many provide similar or identical information (Swenson 2011). We therefore chose to use indices that (i) provided non-redundant information about community turnover and (ii) produced easily comparable measures of alpha and beta diversity, and of standard and phylogenetic diversity. We first calculated measures of within-site diversity (alpha diversity) for each species at each site. For standard alpha diversity analysis, we used the Simpson index, which takes both species richness and evenness into account and weights common species more heavily (Magurran & McGill 2011). We calculated the Simpson index in the vegan package for R (Oksanen *et al.* 2013) as:

$$D = \sum p_i^2 \qquad \text{eqn 1}$$

where D is diversity and p_i is the proportional abundance of species i. We report the 1-D index so that larger values reflect greater diversity.

We next calculated two metrics of phylogenetic alpha diversity for each host species at each site: NRI (net relatedness index) and NTI (nearest taxon index). NRI and NTI are standardized effect size metrics of the mean pairwise phylogenetic distances (MPD) and mean nearest taxon distances (MNTD), respectively, between all individuals in a community (Webb 2000; Webb *et al.* 2002). These indices are complementary, as NRI is better at detecting clustering throughout the phylogenetic tree, whereas NTI detects clustering at the tips of the tree (Vamosi *et al.* 2009; Swenson 2011). Additionally, standardized effect size metrics allow for significance testing and comparisons among studies. NRI was calculated in the picante package for R (Kembel *et al.* 2010) as:

$$NRI = -1(((\overline{X}_{obs}) - \overline{X}(n))/\sigma X(n)) \qquad \text{eqn 2}$$

where $\overline{X}_{obs}$ is the phylogenetic distance between two taxa (i.e. the sum of all intervening branch lengths), and the mean is calculated as the mean of all possible pairs in n taxa. $\overline{X}(n)$ and $\sigma\ X(n)$ are the mean and standard deviation expected for n randomly distributed taxa in the phylogeny pool (Webb *et al.* 2002). NTI was calculated as:

$$\mathrm{NTI} = -1(((\overline{Y}_{obs}) - \overline{Y}(n))/\sigma Y(n)) \qquad \text{eqn 3}$$

where Y_{obs} is the phylogenetic distance to the nearest taxon, and $\overline{Y}_{obs}$, $\overline{Y}(n)$ and $\sigma Y(n)$ are calculated as for NRI (Webb *et al.* 2002).

Significance of NRI and NTI were determined by identifying departures of MPD and MNTD (based on 10 000 permutations) from a null model that shuffled taxon labels on the tips of the phylogeny across all host individuals, resulting in randomized relationships among species (Webb *et al.* 2002; Webb, Ackerly & Kembel 2008). Positive values of NRI and NTI reflect higher than expected community relatedness, or phylogenetic clustering, and negative values indicate phylogenetic overdispersion (Webb *et al.* 2002). We also used the nodesig function in Phylocom v. 4.2 (Webb, Ackerly & Kembel 2008) to determine whether any nodes on the malaria phylogenetic tree had greater or fewer terminal taxa than expected based on random draws from a null model; this effectively asks whether any clades were significantly strain-rich or strain-poor.

To quantify changes in the malaria strain community, we calculated metrics of beta diversity for each combination of host species and site (two host species at three sites, resulting in 15 pairwise comparisons of parasite communities). As with the alpha diversity analysis, we chose easily interpretable indices of beta diversity that provided non-redundant information about phylogenetic turnover. Commonly used beta diversity indices fall into two categories: indices that detect terminal turnover, or turnover at the tips of the phylogenetic tree; and indices that detect basal turnover, or turnover near the base of the phylogenetic tree (Swenson 2011). There are strong correlations between metrics within each group, but basal and terminal indices are complementary (Swenson 2011). Comparing metrics from the two categories can help detect turnover at different phylogenetic scales (e.g. whether turnover is between clades or species within clades).

We used one basal metric and one terminal metric to measure community turnover. We chose β-NRI (also termed D_{pw}) and β-NTI (also termed D_{nn}) because they are directly comparable to the alpha diversity metrics NRI and NTI, but measure phylogenetic distances between samples rather than within samples (eqns 4 and 5; Webb, Ackerly & Kembel 2008). Like NRI and NTI, these are standardized effect size metrics, and significance was determined by calculating deviations of community MPD and MNTD from an abundance-weighted null model that shuffled taxon labels on the phylogenetic tree 999 times. By shuffling relationships among strains and holding all other sources of variance constant, this null model controls for any spatial variation in beta diversity not due to the phylogenetic relationships among strains (i.e. community beta diversity, variation in richness and variation in abundance; Webb *et al.* 2002; Webb, Ackerly & Kembel 2008). β-NTI and β-NRI were calculated in picante (Kembel *et al.* 2010) and Phylocom v. 4.2 (Webb, Ackerly & Kembel 2008) as:

$$\beta - \mathrm{NRI} = \frac{\sum_{i=1}^{nk_1} f_i \overline{X_{ik_2}} + \sum_{j=1}^{nk_2} f_j \overline{X_{jk_1}}}{2} \qquad \text{eqn 4}$$

where $\overline{X_{ik_2}}$ is mean pairwise phylogenetic distance between species i in community k_1 to all species in community k_2, and $\overline{X_{jk_1}}$ is the mean pairwise phylogenetic distance between species j in community k_2 to all species in community k_1, and f_i and f_j are the abundances of species i and j (Swenson 2011; Swenson *et al.* 2012).

$$\beta - \mathrm{NRI} = \frac{\sum_{i=1}^{nk_1} f_i \min Y_{ik_2} + \sum_{j=1}^{nk_2} f_j \min Y_{jk_1}}{2} \qquad \text{eqn 5}$$

where $\min Y_{ik_2}$ is the nearest phylogenetic neighbour of species i from community k_1 to community k_2, and $\min Y_{jk_1}$ is the nearest phylogenetic neighbour of species j from community k_1 to community k_2, and f_i and f_j are the relative abundances of species i and j (Swenson 2011; Swenson *et al.* 2012). Larger values of both indices reflect greater turnover in the community.

We used the abundance-weighted version of the Sørensen dissimilarity index (also called the Bray–Curtis index, eqn 6) to calculate non-phylogenetic beta diversity (hereafter 'community beta diversity'). This index uses numbers of shared and unique species between two communities to quantify differences in community composition; larger values reflect more turnover, or greater dissimilarity, between groups. We chose this metric because simulations show that abundance-weighted phylogenetic metrics such as β-NRI and β-NTI will converge on Sørensen's values when phylogenetic relationships among species are equal (Swenson 2011); The Sørensen's index was calculated in vegan (Oksanen *et al.* 2013) as:

$$d_{jk} = \frac{\sum_i |x_{ij} - x_{ik}|}{\sum_i (x_{ij} + x_{ik})} \qquad \text{eqn 6}$$

where d_{jk} is the dissimilarity between communities, and x_{ij} and x_{ik} refer to the number of species i in communities j and k.

PARASITE COMMUNITY STRUCTURE AND HOST BIOLOGY

We next asked whether phylogenetic and community beta diversity change most with host species, geographical distance or host genetic structure. We used Wilcoxon signed-rank tests to compare estimates of parasite beta diversity between different populations of the same host and of different hosts. We then used Mantel tests with 999 permutations to calculate the correlation between beta diversity matrices and geographical distance between study sites; if parasite communities vary most among geographically distant sites, we expect a positive correlation between distance and beta diversity. Finally, we used partial Mantel tests to determine the correlation between pairwise F_{ST} (microsatellite distance between host populations) and beta diversity estimates, while holding geographical distance constant. Strong correlations between F_{ST} and beta diversity would suggest that the most genetically differentiated host populations have the most unique parasite communities.

Results

PHYLOGENETIC RELATIONSHIPS AMONG STRAINS

We found 131 malaria infections comprising 17 different haplotypes (Fig. 2). Four of these strains had exact

matches in GenBank; all others could be assigned to *Plasmodium* (eight strains) or *Haemoproteus* (nine strains; Fig. 1 inset, Fig. 2). Average sequence divergence between strains was 4·34% (range: 0·2–7%) in *Haemoproteus* and 5·6% (range: 0·2–8·8%) in *Plasmodium*. Five strains were unique to *P. humei,* five unique to *P. trochiloides*, and six were found in both host species (Figs 1 and 2). A single *Haemoproteus* strain, HAE1, only infected *P. humei* and was responsible for 50–75% of infections among sites; there was no similarly dominant strain in *P. trochiloides*. The four strains with exact matches in GenBank are from widely separated geographical locations and different host species, suggesting that many of the strains of avian malaria we identified are cosmopolitan.

HOST GENETIC STRUCTURE

STRUCTURE analyses indicate that the three populations of *P. trochiloides* and *P. humei* we sampled do not correspond to three genetic populations; in both species, the most likely number of population clusters was k = 2 (Fig. S1). However, population subdivisions were not the same across the species: STRUCTURE clustered Indian and Kyrgyz populations of *P. humei* into a single population, with Siberia as a separate cluster, whereas Kyrgyz and Siberian populations of *P. trochiloides* were grouped together, with India as a separate cluster. Inspection of STRUCTURE plots suggests that the Kyrgyz population of *P. humei* is intermediate between India and Siberia, whereas the Indian population of *P. trochiloides* is quite distinct from the Kyrgyz/Siberian populations (Fig. S1). Estimates of F_{ST} are generally consistent with these clusters: pairwise F_{ST} values are small but significantly different among all populations of both species, but are largest between Siberia and India/Kyrgyzstan in *P. humei* and between India and Kyrgyzstan/Siberia in *P. trochiloides* (Fig. S1). The results for *P. trochiloides* are consistent with previous analyses, which indicate that Indian and Kyrgyz-Siberian populations comprise different mitochondrial clades (Irwin *et al*. 2005).

VARIATION IN PREVALENCE

The GLM that best explained variance in infection prevalence included significant main effects of host species and strain genus, and significant interactions between (i) host species and strain genus and (ii) location and strain genus (Fig. 3). More *P. trochiloides* (53/199) were infected with malaria parasites overall than *P. humei* (78/355; main effect of host species: $z = -2{\cdot}97$, $P = 0{\cdot}003$), and there were fewer overall *Plasmodium* infections than *Haemoproteus* infections (main effect of strain genus: $z = 5{\cdot}05$, $P < 0{\cdot}001$). However, prevalence of the different malaria genera differed among host species and sites. *P. humei* were more frequently infected with *Haemoproteus* (host × genus interaction, $z = 5{\cdot}89$, $P < 0{\cdot}001$; Fig. 3). A higher incidence of *Plasmodium* infections in both host species in Siberia meant that *Plasmodium* showed greater variation among sites than *Haemoproteus* (genus × site interaction: $z = 2{\cdot}40$, $P = 0{\cdot}016$; Fig. 3).

We also conducted separate analyses for each host species. In *P. trochiloides*, the final model included a significant effect of site, due to low infection prevalence in Kyrgyzstan (Tukey post hoc test, $z = -2{\cdot}34$, $P = 0{\cdot}05$) and a marginally non-significant effect of strain type, with a slightly higher incidence of *Plasmodium* than *Haemoproteus* infections ($z = 1{\cdot}68$, $P = 0{\cdot}09$). In *P. humei*, there are many fewer *Plasmodium* than *Haemoproteus* infections ($z = 3{\cdot}19$, $P = 0{\cdot}001$), and a significant site by strain interaction due to comparatively high *Plasmodium* prevalence in Siberia ($z = 2{\cdot}04$, $P = 0{\cdot}04$). Taken together, these results indicate that Siberian birds of both species have more *Plasmodium* infections, and that *P. trochiloides* experiences more geographical variation in malaria infections than *P. humei* (Fig. 3).

We found no infections in a subset of 10-day-old chicks ($n = 40$), and chicks were consequently excluded from all analyses. This may be because malaria is sometimes not detectable until 14 days post-infection (Valkiūnas 2005), and our sampling occurred at 10 days post-hatching, or it might indicate that transmission of malaria infections does not occur on the breeding grounds.

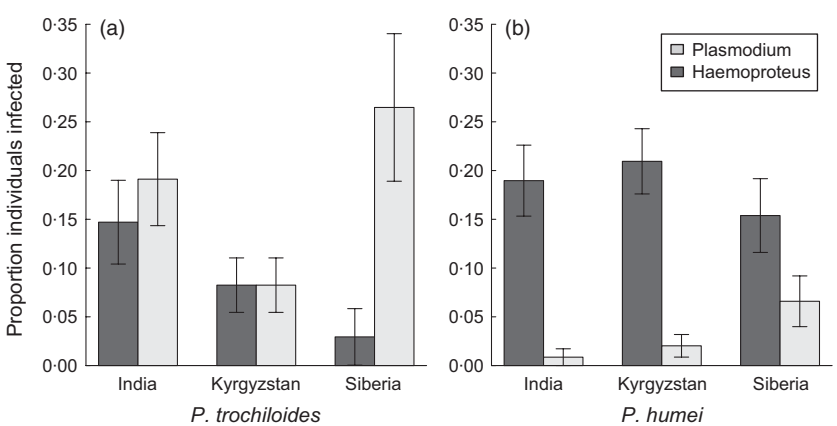


Fig. 3. Variation in the prevalence of *Haemoproteus* (dark grey) and *Plasmodium* (light grey) infections in a) *P. trochiloides* and b) *P. humei* at each of the three study sites. Bars (±SE) show the proportion of individuals screened that were infected by each of the two parasite genera. There are significantly more *Plasmodium* infections in *P. trochiloides* and more *Haemoproteus* infections in *P. humei*. There is also a significant strain genus by site interaction due to higher incidence of *Plasmodium* infections in Siberia.

Table 1. Measures of parasite community diversity within each species and site. Significant values ($P < 0{\cdot}05$) in relation to a null model are boldfaced. Simpson = Simpson's diversity index, NRI = net relatedness index, NTI = nearest taxon index. Larger values of Simpson's index indicate greater diversity. Larger values of NRI and NTI indicate phylogenetic clumping; negative values indicate phylogenetic evenness. There is evidence for significant phylogenetic clumping in *P. humei* in India; all other communities are structured randomly with respect to phylogeny

Community	N taxa	Simpson	NRI	NTI
P. humei India	8	0·556	**2·3313**	0·9886
P. humei Kyrgyzstan	5	0·465	0·165	−0·1436
P. humei Siberia	7	0·700	−0·2345	−0·0806
P. trochiloides India	8	0·836	−0·6019	0·4141
P. trochiloides Kyrgyzstan	7	0·828	−0·5749	0·2653
P. trochiloides Siberia	3	0·540	−0·3162	−0·6083

VARIATION IN ALPHA DIVERSITY

Analyses of community alpha diversity using Simpson's index indicate generally higher parasite strain diversity in *P. trochiloides* compared to *P. humei* (Table 1, Figs 1 and 2). However, malaria communities in *P. trochiloides* show no apparent signature of phylogenetic processes; NRI and NTI were not significant in any *P. trochiloides* populations (Table 1). Conversely, there is evidence for some phylogenetic structuring in *P. humei* parasite communities. We found significant positive NRI, and marginally non-significant positive NTI, in Indian populations of *P. humei* (Table 1), indicating that parasite strains in this host population are phylogenetically clustered and that clustering is relatively basal in the malaria phylogenetic tree (given the stronger effect of NRI vs. NTI). The only node in the malaria phylogeny exhibiting evidence of greater than expected diversification in the nodesig analysis was the basal node of the *Haemoproteus* clade (Fig. 2). Together, these results suggest that phylogenetic conservatism has led to a diverse community of *Haemoproteus* strains in the two host species, particularly in Indian *P. humei*.

VARIATION IN BETA DIVERSITY

Like the phylogenetic alpha diversity metrics, analyses of phylogenetic beta diversity indicate phylogenetic conservatism of strain communities in *P. humei*, with significantly lower β-NTI values and marginally non-significant β-NRI values, between Indian and Kyrgyz populations of *P. humei,* compared to the null model (Table 2). Thus, the *Haemoproteus*-dominated communities in *P. humei* exhibit phylogenetic clustering in the Indian population (Table 1), as well as less phylogenetic turnover than expected with the neighbouring Kyrgyz population (Table 2). Conversely, in *Plasmodium*-dominated *P. trochiloides,* there is no significant signal of β-NRI or β-NTI in any pairwise comparisons, despite relatively high community turnover as measured by the Sørensen's dissimilarity index (Table 2).

In summary, we found relatively little evidence for a phylogenetic signal in the spatial structure of malaria communities in either of the two host species. This interpretation is bolstered by a significant correlation between community (Sørensen) and phylogenetic (β-NRI, β-NTI) metrics of beta diversity (partial Mantel test with Euclidean distance between sites held constant: Sørensen- β-NRI, $r = 0{\cdot}833$, $P = 0{\cdot}007$; Sørensen- β-NTI, $r = 0{\cdot}812$, $P = 0{\cdot}002$). Non-phylogenetic metrics of beta diversity thus indicate similar patterns of malaria community turnover within and among host species as do phylogenetic metrics.

Table 2. Measures of community turnover. Above the diagonal: β-NRI values. Below the diagonal: pairwise estimates of Sørensen's dissimilarity index. Larger numbers reflect greater differences in community structure (i.e. greater turnover). Within-species comparisons are in italics. Above the diagonal, the significant pairwise comparison is boldfaced. Turnover in parasite communities among populations of the same species is less than among populations of different species, even among those occupying the same breeding site. In general, there is no strong imprint of phylogeny

	India *P. humei*	Kyrgyzstan *P. humei*	Siberia *P. humei*	India *P. trochiloides*	Kyrgyzstan *P. trochiloides*	Siberia *P. trochiloides*
India *P. humei*	–	**0·061**	0·094	0·149	0·138	0·187
Kyrgyzstan *P. humei*	0·404	–	0·100	0·149	0·139	0·184
Siberia *P. humei*	0·442	0·481	–	0·148	0·145	0·164
India *P. trochiloides*	0·826	0·719	0·860	–	0·144	0·140
Kyrgyzstan *P. trochiloides*	0·795	0·800	0·833	0·590	–	0·143
Siberia *P. trochiloides*	0·939	0·909	0·867	0·758	0·462	–

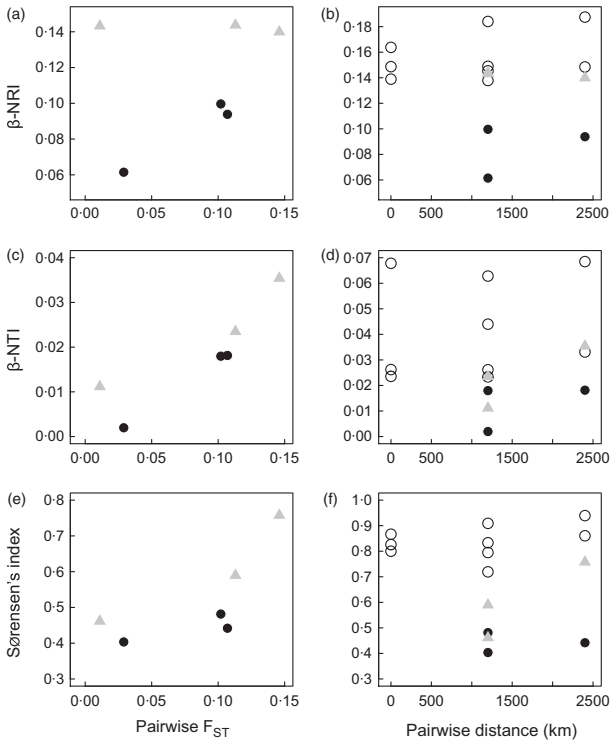


Fig. 4. Relationship between three metrics of parasite beta diversity, host pairwise F_{ST} (a,c,e) and host geographical distance (b,d, f). Larger values on the *y*-axis for all three diversity metrics represent greater turnover in the parasite community. For F_{ST}, plotted values are within-host pairwise comparisons among the three sites for *P. trochiloides* (▲) and *P. humei* (●). For geographical distance, between-host turnover in parasite communities (○) is plotted, as well as within-host community turnover for *P. trochiloides* (▲) and *P. humei* (●). Populations of the different host species inhabiting the same site are given a geographical distance equal to zero. Panels (a,c,e): there is no significant relationship between any measure of community turnover and genetic distance between host populations. Panels (b,d,f): for all metrics of beta diversity there is more turnover in parasite communities among hosts, even those inhabiting the same site, than there is among the same host occupying different sites.

PARASITE COMMUNITY STRUCTURE AND HOST BIOLOGY

Finally, we asked whether different metrics of parasite beta diversity were related to aspects of host biology, specifically study site, geographical distance between host populations, or genetic distance between host populations. We examined the effect of study site by comparing within-host estimates of beta diversity to among-host estimates for each of the two host species. As suggested by the difference in the prevalence of *Haemoproteus* and *Plasmodium*, we found less turnover in parasite communities (smaller values of beta diversity) between different populations of the same host than between populations of different hosts, even those breeding at the same site. This indicates a stronger effect of host than site on parasite community structure. In *P. humei,* this pattern is significant across all three measures of beta diversity (Wilcoxon signed-rank test: $W = 0$, $P = 0{\cdot}009$ for β-NRI, β-NTI, and Sørensen's, Fig. 4). In *P. trochiloides,* there was significantly less turnover within- than between-host species in estimates of community beta diversity (Wilcoxon signed-rank test, $W = 1$, $P = 0{\cdot}018$, Fig. 4), but no significant differences in within- vs. between-host comparisons for estimates of phylogenetic beta diversity (Wilcoxon signed-rank test, $W = 6$ $P = 0{\cdot}291$ for β-NRI, β-NTI, Fig. 4), again indicating that phylogenetic processes do not appear to play an important role in structuring parasite communities within *P. trochiloides*.

We next quantified effects of geographical distance *per se* on turnover of parasite communities by considering host species occupying the same site to have a distance of zero, India–Kyrgyzstan and Kyrgyzstan–Siberia to have distances of 1200 km, and India–Siberia to have a distance of 2400 km. We found no correlation between distance and any measure of beta diversity (Mantel tests: β-NRI, $r = 0{\cdot}098$, $P = 0{\cdot}544$; β-NTI, $r = -0{\cdot}027$, $P = 0{\cdot}454$; Sørensen, $r = -0{\cdot}176$, $P = 0{\cdot}659$, Fig. 4), again implying that different host species occupying the same site do not have more similar parasite communities than more distant populations. Finally, we found no correlation in either host species between F_{ST} and any measure of beta diversity when distance between sites was held constant using partial Mantel tests (Fig. 4), although sample sizes for these comparisons were small.

Discussion

The ecological factors that influence the structure of micro-organism communities remain poorly understood. In this study, we asked if host species, study site and/or geographical and genetic distance between host populations could explain variation in prevalence and community structure of avian malaria. We found that parasite communities are more similar among populations of the same host than among sympatric populations of different, albeit closely related, host species. Moreover, we found no effects of host genetic structure or geographical distance on malaria communities.

PREVALENCE AND PHYLOGENETIC CLUSTERING OF PARASITES WITHIN HOSTS

Overall incidence of malaria infection was similar in *P. trochiloides* and *P. humei*, but the prevalence of two malaria genera (*Haemoproteus* and *Plasmodium*) varied between host species. In all populations, *P. trochiloides* was infected more often with *Plasmodium*, whereas *P. humei* was primarily infected with *Haemoproteus*. Moreover, metrics of phylogenetic alpha diversity indicate different patterns of diversification for the two malaria genera. The *Haemoproteus* clade has diversified more than expected, and *Hameoproteus*-dominated communities in Indian *P. humei* show evidence of phylogenetic clustering. There is also less phylogenetic turnover

at the base of the tree than expected between the malaria communities in Indian and Kyrgyz *P. humei*, indicating that differences between neighbouring communities are in different lineages of *Haemoproteus* rather than different genera of malaria. By contrast, although the *Plasmodium*-dominated communities in *P. trochiloides* exhibit approximately the same amount of turnover between host populations as do *P. humei* communities, there is no phylogenetic signature to this turnover. Malaria in *P. trochiloides* thus seems to be structured by factors unrelated to the phylogenetic relationships among strains.

These different patterns of community and phylogenetic structure could reflect differences in parasite and/or host ecology. For example, the different life histories of *Haemoproteus* and *Plasmodium* parasites may influence their relative diversification within host species. *Haemoproteus*, while more common in birds (and in our two host species), tends to be more specialized than *Plasmodium* (Valkiūnas 2005). The lack of phylogenetic signal in *P. trochiloides* communities could be because *Plasmodium* strains infect other hosts in the community, resulting in little signal of parasite diversification within a single host (Poulin, Krasnov & Mouillot 2011). Conversely, the phylogenetic clustering observed in *Haemoproteus* strains within *P. humei*, as well as the dominance of one strain across many populations (Fig. 1), may reflect specialization of this group of parasites within a single host, although more host species would need to be sampled to confirm this possibility.

Host ecology could also influence differences in parasite phylogenetic clustering. *P. trochiloides* and *P. humei* are both migratory species that have expanded their historical Himalayan breeding ranges north into Russia since the last glacial maximum (Irwin *et al.* 2001, 2005). Lower phylogenetic diversity in northern parasite communities may be a signature of these recent range expansions, as there has been less time for host populations to accumulate new parasite lineages. Although we found no evidence for variation in phylogenetic alpha diversity (NRI and NTI) among populations of *P. trochiloides*, range expansions could explain the comparatively more diverse and phylogenetically clustered *Haemoproteus* communities in older Indian (i.e. southern) populations of *P. humei*.

Finally, variation in *Haemoproteus* and *Plasmodium* infections could be related to the distribution of their vectors, particularly if different vectors prefer different host species or hosts differ in their immune responses to different vectors. *Plasmodium* is transmitted via mosquito, and there are more mosquitoes in Siberia compared to the other sites (E. Scordato, pers. obs.); there were also more *Plasmodium* infections in both host species in Siberia. However, mosquitoes in Siberia can only affect infection prevalence if malaria transmission occurs on the breeding grounds. Variation in malaria diversity could also be influenced by differences in transmission grounds among the two host species, which we consider below.

EFFECTS OF HOST HABITAT, PHYLOGEOGRAPHY AND TRANSMISSION GROUNDS ON PARASITE BETA DIVERSITY

Consistent with *Haemoproteus* and *Plasmodium* infecting the two host species at different rates, all beta diversity metrics indicate that parasite communities are more similar across populations of the same host species than between different hosts in the same breeding habitat. There are also no significant effects of geographical distance between host populations on parasite beta diversity, providing no support for a distance–decay relationship in this system. These malaria communities thus do not consistently turnover with latitude, and, from the perspective of host evolution, birds breeding in different areas do not encounter radically different parasite communities. Moreover, we find no significant relationship between malaria communities and host genetic structure, indicating that more divergent host populations do not have more divergent parasite communities.

The lack of distance–decay patterns of geographical variation, the different rates of infection of *Haemoproteus* and *Plasmodium* in the host species and the absence of infected chicks suggest that malaria transmission in *P. humei* and *P. trochiloides* may occur primarily in the winter or during migration rather than on the breeding grounds. Moreover, during the breeding season, there are no obvious microhabitat differences between host species that could contribute to differences in vector exposure and thereby prevalence of *Haemoproteus* vs. *Plasmodium* infections: in addition to breeding sympatrically, the host species are morphologically similar insectivores that often forage in the same trees and eat similarly sized prey (Scordato 2012). However, the birds are parapatric in winter, overlapping only in central India (Gross & Price 2000). Variation in vector exposure on different wintering grounds could produce the observed differences in infection rates for different malaria genera, as well as geographical variation in strain communities among breeding populations.

Transmission grounds can have profound effects on host–parasite dynamics. For example, the phylogenetic clustering we observe in *Haemoproteus* mirrors a continental pattern: Hellgren *et al.* (2007) reconstructed the transmission grounds for over 200 malaria strains in Europe and Africa and found a strong phylogenetic signal of transmission area (winter vs. breeding) in *Haemoproteus* but not *Plasmodium*, suggesting that infrequent dispersal has produced phylogenetic clustering of lineages in different transmission regions. Conservation of transmission grounds and infrequent switching between resident and migrant host species could facilitate the evolution of closely related strain communities within hosts (Ricklefs & Fallon 2002; Hellgren *et al.* 2007).

IMPLICATIONS FOR PARASITE COMMUNITY ECOLOGY AND HOST–PARASITE INTERACTIONS

Previous studies of beta diversity in avian malaria found some instances of distance–decay relationships between parasite communities, but also found variation that could not be explained by host genetic structure, geographical distance or vector diversity (Ishtiaq *et al.* 2009; Svensson-Coelho & Ricklefs 2011). We added a phylogenetic component to analyses of malaria beta diversity, and while we find some evidence for phylogenetic clustering in one population, like other studies, we find little evidence for host phylogeography or distance–decay processes influencing parasite communities. Instead, it seems likely that variation due to host species, and possibly transmission location and/or parasite life history, contribute to the geographical variation in parasite prevalence and community structure we observe in this study.

The ecological and evolutionary processes that affect parasite communities can also shape host communities. For example, aspects of host ecology, such as population density and body mass, have been linked to endoparasite community richness (e.g. Arneberg 2002). Hosts exposed to more diverse parasite communities can experience greater fitness costs (Arriero & Møller 2008) and may need to mount different immune responses to more distantly related parasites compared to closely related strains. Determining how genetic variation among different malaria strains influences community structure and host ecology remains an important avenue for future research. Although there are likely thousands 'species' of avian malaria (Bensch *et al.* 2000), it is not known how different strains differ in virulence, or how the phylogenetic structure of malaria communities affects host ecology and fitness. In this study, we treated each malaria haplotype as a unique strain, but some of these may be variants of the same species. Nonetheless, we have shown that metrics of phylogenetic and community diversity can be powerful tools for assessing structure and turnover in avian malaria communities. Applying these metrics to a broader sampling of host species will help resolve the role of evolutionary relationships among parasite strains in structuring both host and parasite communities. Future work correlating turnover in parasites with other aspects of ecology, such as vector abundance and host population density, will shed light on the factors influencing variation in parasite communities and their broader ecosystems.

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Data accessibility

Data are available on Dryad (doi:10.5061/dryad.q1431) and GenBank (Accession numbers KJ396623-KJ396639).

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Supporting Information

Additional Supporting Information may be found in the online version of this article.

Fig. S1. STRUCTURE plot for *Phylloscopus humei* and *Phylloscopus trochiloides* with F_{ST} data.

Appendix S1. Protocol for development of new microsatellite loci, identification of infected individuals, construction of phylogenetic tree, and analysis of host population structure.

Table S1. Primer sequences and diagnostic information for eight new microsatellite loci in *P. trochiloides*