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# Infection dynamics of endemic malaria in a wild bird population: parasite species-dependent drivers of spatial and temporal variation in transmission rates

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## Summary

**1.** Investigating the ecological context in which host–parasite interactions occur and the roles of biotic and abiotic factors in forcing infection dynamics is essential to understanding disease transmission, spread and maintenance.

**2.** Despite their prominence as model host–pathogen systems, the relative influence of environmental heterogeneity and host characteristics in influencing the infection dynamics of avian blood parasites has rarely been assessed in the wild, particularly at a within-population scale.

**3.** We used a novel multievent modelling framework (an extension of multistate mark–recapture modelling) that allows for uncertainty in disease state, to estimate transmission parameters and assess variation in the infection dynamics of avian malaria in a large, longitudinally sampled data set of breeding blue tits infected with two divergent species of *Plasmodium* parasites.

**4.** We found striking temporal and spatial heterogeneity in the disease incidence rate and the likelihood of recovery within this single population and demonstrate marked differences in the relative influence of environmental and host factors in forcing the infection dynamics of the two *Plasmodium* species.

**5.** Proximity to a permanent water source greatly influenced the transmission rates of *P. circumflexum*, but not of *P. relictum*, suggesting that these parasites are transmitted by different vectors.

**6.** Host characteristics (age/sex) were found to influence infection rates but not recovery rates, and their influence on infection rates was also dependent on parasite species: *P. relictum* infection rates varied with host age, whilst *P. circumflexum* infection rates varied with host sex.

**7.** Our analyses reveal that transmission of endemic avian malaria is a result of complex interactions between biotic and abiotic components that can operate on small spatial scales and demonstrate that knowledge of the drivers of spatial and temporal heterogeneity in disease transmission will be crucial for developing accurate epidemiological models and a thorough understanding of the evolutionary implications of pathogens.

**Key-words:** Avian malaria, blue tits, *Cyanistes caeruleus*, disease incidence rate, environmental heterogeneity, host age, host sex, multievent mark–recapture models, *Plasmodium*, transition rates

## Introduction

Transmission is a key epidemiological process for understanding host–pathogen dynamics and predicting the effects of disease on populations (McCallum, Barlow & Hone 2001). In addition to biotic factors, such as host age, sex or abundance, parasite transmission in natural populations may be influenced by abiotic factors, such as microclimate and landscape (Hudson *et al.* 2002). For example, transmission of vector-borne pathogens will be at least partly governed by environmental traits that limit vector abundance and the spatial and temporal distribution of vectors (Sinski *et al.* 2006; Byers *et al.* 2008). Because transmission processes can be proximately driven by local conditions, spatial heterogeneity

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in environmental factors will play an important role in mediating infection dynamics and the spread and persistence of disease (Byers *et al*. 2008; Osnas *et al*. 2009).

In addition to effects on transmission, environmental heterogeneity may also alter various components of host fitness. For example, host condition, nutritional status or stress level may vary with habitat quality resulting in differential impacts of pathogens over relatively small spatial scales (Lafferty & Kuris 1999; Beldomenico *et al*. 2009). Hence, the environment in which hosts and parasites interact can also substantially affect the strength and specificity of selection (Wolinska & King 2009). Ultimately, spatial variability in the drivers of host infection and in pathogen-induced selection pressure on hosts will direct patterns of local adaptation and co-evolutionary processes (Kaltz & Shykoff 1998; Dybdahl & Storfer 2003). Thus, investigating the ecological context in which host–parasite interactions occur and the roles of biotic and abiotic factors in forcing infection dynamics is essential to understanding the dynamics of disease spread and maintenance in the wild and the evolutionary implications of parasites for hosts. Such knowledge is particularly relevant in a world facing significant climate and anthropogenic change but has received scant attention for most wildlife diseases (Daszak, Cunningham & Hyatt 2001; Harvell *et al*. 2009).

Avian malaria (*Plasmodium* or *Haemoproteus* spp. Valkiūnas 2005) are globally distributed vector-borne parasites commonly used as model systems for testing hypotheses in evolutionary ecology (Ricklefs, Fallon & Bermingham 2004; Knowles, Nakagawa & Sheldon 2009), and investigating diagnostic traits and control options for human malaria (Slater 2005). Studies of human malaria (*P. falciparum*) have revealed transmission rates to be largely governed by environmental factors, such as altitude and proximity to water, which restrict the distribution of mosquito vectors (Foley *et al*. 2003; Balls *et al*. 2004). It is likely, however, that host demographic factors could play a more significant role in the transmission of avian relative to human malaria because synchronized breeding seasons generate periodic recruitment of immunologically naive juveniles to host populations and because disparity in reproductive behaviours of male and female birds may differentially affect their exposure to vectors (Arriero & Moller 2008; Cosgrove *et al*. 2008). Although correlations between host prevalence and environmental variables have been documented for avian malaria species at a range of spatial scales (Atkinson *et al*. 2005; Freed *et al*. 2005; Wood *et al*. 2007; O'Connor, Dudaniec & Kleindorfer 2010), variation in population prevalence may not necessarily reflect variation in the underlying transmission rates (Anderson & May 1979; Bolzoni, Real & De Leo 2007). Moreover, prevalence estimates may be subject to significant bias if the detection probabilities for infected and uninfected individuals in different landscapes vary (Jennelle *et al*. 2007).

Quantifying transmission rates of avian malaria, like many wildlife diseases, is difficult because, unlike for human diseases, individuals cannot all be counted or examined, and contact tracing (as is done for human diseases, such as SARS, see Lipsitch *et al*. 2003) is essentially impossible (McCallum, Barlow & Hone 2001; Caley & Hone 2004). Multistate mark–recapture models provide a framework for estimating epidemiologically relevant transmission parameters in natural populations, whilst explicitly accounting for variability in detection rates with infection status (Atkinson & Samuel 2010, Conn & Cooch 2009, Faustino *et al*., 2004, Schwarz, Schweigert & Arnason 1993). Transition rates obtained from multistate models are a compound measure of the probability of becoming infected and surviving to be captured and thus provide conservative estimates of infection rates, recovery rates and rates of change between different infections. In particular, transitions to infected states provide a conservative estimate of disease incidence rate (the discrete probability that susceptible individuals becoming infected during time $i$ to $i + 1$, conditional on survival, Atkinson & Samuel 2010), which are related to the force of infection in the population (the rate at which susceptible hosts acquire infections, Heisey, Joly & Messier 2006; Ozgul *et al*. 2009). Although fast becoming an integral tool in wildlife disease ecology, such models have rarely been used to assess infection dynamics in avian malaria systems (Atkinson & Samuel 2010).

Another consideration in understanding malaria transmission in the wild is the variety of malaria species that may comprise infections in host populations (Bensch *et al*. 2004; Waldenström *et al*. 2004). Because both the prevalence and the distribution of different species may be governed by contrasting environmental conditions (Wood *et al*. 2007) and because different species might have quite different virulence in a given host (Lachish *et al*. 2011), species diversity can constitute a potentially important source of variation in risk of exposure and infection for hosts. To date, very few ecological studies of malaria have considered this diversity of malaria species with respect to infection dynamics.

In this study, we used multistate mark–recapture models to assess variation in infection dynamics of malaria in a long-term monitored population of blue tits (*Cyanistes caeruleus*) infected with two divergent *Plasmodium* species (*P. relictum* and *P. circumflexum*, Valkiūnas 2005). In a companion paper, we report the results of survival and recapture rate modelling from these multistate models, showing that these two malaria species impact on host fitness components in contrasting ways (Lachish *et al*. 2011). Here, we report the results of transition rate modelling using the same basic model structure to assess the patterns of infection and recovery rates within the population. Previous work has revealed marked variation in the spatial pattern of prevalence of these two malaria species within this population, in relation to key landscape (proximity to permanent water) and host characteristics (host age and sex, Wood *et al*. 2007). Hence, in this study, we aimed to (i) derive basic estimates of rates of infections and recovery; (ii) assess the role of these biological and environmental factors in forcing transmission processes within this population and (iii) determine whether the influence of biotic and abiotic factors on infection dynamics differs between malaria species.

## Materials and methods

Full details of the field and molecular diagnosis protocols used in this study are given in Lachish *et al.* (2011) along with fuller methods for the mark–recapture analyses. Here, we give brief details of these general methods and describe in full additional methods pertinent to the transition rate analyses presented in this paper.

### STUDY SITE, HOST SPECIES AND AVIAN MALARIA DIAGNOSIS

From 2001 to 2009, blood samples were collected from individually marked blue tits (*Cyanistes caeruleus*) within Wytham Woods, near Oxford, UK (51°46′N, 1°20′W). Blood samples for diagnosis were collected annually between days 6 and 14 of the nestling phase, from parents feeding young in nest boxes. Blood samples were screened for infections of two *Plasmodium* morphospecies (based on cytochrome *b* sequences), *P. relictum* and *P. circumflexum* (Palinauskas *et al.* 2007), that comprise 98·2% of infections in the study population (Knowles *et al.* 2011). Infections were diagnosed either by nested polymerase chain reaction (PCR) assays from 2001 to 2004 (protocols described in Wood *et al.* 2007; Waldenström *et al.* 2004) or by quantitative (q)PCR assays from 2005 to 2009 (protocol details described in Knowles *et al.* 2011). For simplicity, in model notation, we refer to *P. relictum* as R-Clade and *P. circumflexum* as C-Clade (note that this is a change in terminology from previous work, e.g. Wood *et al.* 2007; Cosgrove *et al.* 2008; and reflects the identification of morphospecies that corresponded to distinct mtDNA lineages; Palinauskas *et al.* 2007).

### MSMR MODELLING APPROACH

The mark–recapture data set consisted of yearly capture histories for all breeding birds captured within the study site each breeding season, grouped by sex and age and assigned to different disease states on the basis of their infection status at the time of capture. To incorporate individuals of unknown infection status [those for which blood samples were not obtained at capture, or for which analysis of blood samples was not carried out ($N = 1443$), for which species diagnosis was unresolved ($N = 46$), or for which diagnosis revealed mixed species infections ($N = 52$)] within our multistate mark–recapture framework, we employed newly developed 'multievent' models, an extension of multistate models (Pradel 2005; Conn & Cooch 2009). By explicitly accounting for unknown or partially observable states, by treating them as a hidden Markov process, multievent models allow for uncertainty in the detection of disease state, but not for error in the assignment of disease states. As discussed in Lachish *et al.* (2011), stringent laboratory procedures ensure that false-positive diagnoses will be rare in this study, although false-negative diagnoses may have occurred, as the majority of infections are chronic with low parasitaemia. However, analyses using occupancy modelling have shown the qPCR assay used in this study is highly sensitive, with a very low probability of false-negative diagnoses (S. Lachish, A. M. Gopalaswamy, S. C. L. Knowles & B. C. Sheldon, unpublished data). Moreover, when the probability of false positives is low and the true detection probability is at least 50%, then provided at least three samples are tested per unit (our qPCR diagnostic assays were run in triplicate) there will be very little bias in estimates (McClintock *et al.* 2010). The majority of diagnoses in this study were undertaken with qPCR in which samples were analysed in triplicate. Also, owing to the paucity of information on infection status in the early years of this study (only 58% of captured individuals were tested for malaria in 2001 and no individuals were tested in 2002) only two transition rate estimates were obtained in the years when nested PCR was used. Hence, we believe that the potential for biased estimates to produce spurious inferences in this study is minimal.

We conducted two multievent mark–recapture analyses to assess the infection dynamics of malaria in our study population. In the first analysis, we combined both *Plasmodium* species into a single infected state to assess the general patterns of infection and recovery within the population (see fig. 1a in Lachish *et al.* 2011). Capture histories for this analysis were assigned to one of three events (captured and infected, captured and uninfected, captured but infection status unknown) corresponding to two disease states (infected and uninfected). All state transitions were possible, as infected individuals can recover, and recovered individuals may become re-infected, with yearly infection and recovery rates given by the transitions from uninfected to infected states and from infected to uninfected states, respectively. In the second analysis, we kept both *Plasmodium* species separate to assess species-specific infection dynamics and assigned capture histories to one of four events (captured with R-Clade infection, captured with C-Clade infection, captured and uninfected, captured but infection status unknown), corresponding to three disease states (uninfected, infected with R-Clade, infected with C-Clade; see fig. 1b in Lachish *et al.* 2011). Again all transitions between states were possible, with transition rates now representing species-specific infection and recovery rates and rates of switching between infection types. Because acute malaria infections appear to entail substantial mortality costs for hosts in this population (Lachish *et al.* 2011), transition rates estimated in this study will largely reflect infection dynamics amongst uninfected and chronically infected individuals. In addition, as a consequence of *Plasmodium* epidemiology (Valkiūnas 2005), annual estimates of infection rates will capture both new infections and relapses of previous infections, whilst annual estimates of recovery will comprise both true recovery (sterilizing immunity) and apparent recovery (involving the disappearance of active blood-stage infections when infections remain latent in tissues or are suppressed below the diagnostic detection limit; see also Discussion).

For each analysis, we employed a three-stage model ranking process. We first modelled recapture rates with survival and transition rates fully parameterized (see Lachish *et al.* 2011 for details of the global model). Survival rates were then modelled using the most parsimonious recapture rate model identified in step one. The results of these recapture and survival rate models are reported elsewhere (Lachish *et al.* 2011). Here, we report the final stage in the MSMR model process: modelling variation in transition rates, using the most parsimonious recapture and survival models identified in the first two stages as the base models for this final stage. For the combined *Plasmodium* analysis, the base model for recapture rate was state and sex dependent (St + Sx), for survival rate was age (first year birds vs. adults of 2+ years of age) and sex dependent (a2 + Sx) and allowed for temporal variation in all transition rates as well as age and sex effects [(St*t + a2 + Sx); QAIC = 7525·71; see Table 1 for notation and table 3 in Lachish *et al.* in press for model selection results]. For the separate-species analysis, the base model for recapture rate was state and nest box density-dependent (St + boxD), with survival modelled as state, time and age dependent (St + t + a2) and allowed for temporal variation in all transition rates (except those occurring between R-Clade and C-Clade as these transitions were infrequent in our data set) as well as age and sex effects [(St*t + a2 + Sx); QAIC = 7141·21 see table 4 in Lachish *et al.* 2011]. Whilst the modelling process for this paper is an extension of

**Table 1.** Notation used to denote the main effects and model structure used in modelling transition rates ($\Psi$) of blue tits infected with *Plasmodium* species (*P. relictum* = R-Clade; *P. circumflexum* = C-Clade)

| Model notation | Description |
|---|---|
| St | State-dependent transition rates (indicates that all transition rates differ and covariate effects apply to all transition rates) |
| Inf | Infection rates (indicates that covariate effects are limited to transitions from uninfected to infected states: $\Psi_{UI}$ for the clades combined analysis, or $\Psi_{UR}$ and $\Psi_{UC}$ for the separate clades analysis) |
| - (RInf; CInf) | Clade-specific infection rates (covariate effects limited to $\Psi_{UR}$ or $\Psi_{UC}$, respectively) |
| Rec | Recovery rates (indicates that covariate effects are limited to transitions from infected to uninfected states: $\Psi_{IU}$ for the clades combined analysis, or $\Psi_{UR}$ and $\Psi_{UC}$ for the separate clades analysis) |
| - (RRec; CRec) | Clade-specific recovery rates (covariate effects limited to $\Psi_{RU}$ or $\Psi_{CU}$, respectively) |
| RC | Transition from R-clade infection to C-clade infection |
| CR | Transition from C-clade infection to R-clade infection |
| Covariate effects | |
| Sx | Sex effect |
| a2 | Age effect (yearlings vs. adults) |
| river | Distance from the River Thames (dichotomous near/far covariate) |
| d2river | Distance from the River Thames (continuous individual covariate) |
| t | Time dependence (yearly variation) |
| c | Constant rate (no covariates) |

the previous work, the question (dynamics of infection vs. effects on hosts) is different, and separation of the two allows more detailed dissection of the effects.

To assess the relative influence of environmental and host factors in mediating transmission dynamics and infection risk within the host population, we modelled variation in transition rates in two steps. Based on *a priori* knowledge, we first assessed the importance of temporal and environmental variation relative to constant transition rates by modelling transition rates in relation to time (year) and proximity to a major water body, the River Thames (which are known to be correlated with *Plasmodium* prevalence in this population, Wood *et al.* 2007). Proximity to the river was included in models either as: (i) a dichotomous covariate, with individuals classified as being either near the river (captured in nest boxes that were ≤500 m from the river) or far from the river (captured > 500 m from the river) based on previous work showing higher *Plasmodium* prevalence within this distance of the River Thames (Wood *et al.* 2007); or as (ii) a continuous individual-specific covariate, with the (standardized) distance to the river determined by GIS from the nest box in which individuals were first captured breeding. We investigated the additive and two-way interactive effects of time and river on state transitions (including state*time and state*river interactions with transition rates only). However, as mentioned earlier, in the species-specific analysis, changes in infection status from R-Clade to C-Clade infection or vice versa were constrained to be time-invariant, as these transitions were infrequent in our data set. Also, to limit the number of candidate models in the species-specific analysis, the 'continuous' river covariate was only modelled as an alternative for the 'dichotomous' river effect in the best identified model (and was not included in further models, as it did not improve the fit of this model, see Results). In the second step, we assessed variation in transition rates in relation to host sex and age, whilst retaining the temporal and river effects of the best model in the previous step. We investigated the additive and two-way interactive effects of age and sex on state transitions (including state*age and state*sex interactions only). Note that in the species-specific analysis, the effects of host age and sex were not modelled for transitions that occurred between R-Clade and C-Clade infections.

All models were fitted to the data using program E-SURGE (Choquet 2009) using the general model structure described in Conn & Cooch (2009; specific details given in Lachish *et al.* 2011). Model selection was based on small sample size corrected Akaike Information Criteria adjusted for overdispersion (QAICc with $\hat{c} = 1{\cdot}5$; see Lachish *et al.*, 2011, for details of goodness-of-fit procedures for global models). ESURGE automatically adjusts AIC values and parameter variances to account for this variance inflation factor. The relative likelihood of each model in the candidate set was estimated with normalized QAICc *w*eights ($w_i$, or the index of relative plausibility). Model notation is explained in Table 1.

## Results

A total of 3424 birds were captured an average of 1·4 times for a total of 4843 captures over the 9 years of the study (complete summaries of mark–recapture data provided in Appendix S1). As no information on infection status was available for individuals captured in 2002, transition rates between all states in both analyses were inestimable in the early years of the study [from 2001 to 2003; confidence intervals for these estimates were either abnormally large (from 0 to 1) or small (equal to zero)]. As these early years contributed to robust inferences of survival and recapture rates in this population (see Lachish *et al.* 2011), they were retained in the model structure, and, however, their exclusion did not qualitatively change results. From 2004 to 2009, we observed 175 transitions between known disease states in the combined-species analysis and 167 transitions amongst known disease states (the uninfected and the two infected states) in the species-specific analysis (all transitions including those involving unknown disease states were more numerous: 381 for the combined analysis and 354 for the clade-specific analysis).

The combined-species analysis revealed very strong support for models in which transition rates varied between years and in relation to whether individuals were near or far from the river (i.e. treated as a dichotomous variable), indicating that both the disease incidence rate and the likelihood of recovery vary temporally within the population, and

**Table 2.** Summary results of the multievent mark–recapture analysis modelling transition rates of combined *Plasmodium* infections in blue tits. The top 10 models in each modelling step are shown (as well the model with constant transition rates). Recapture rate was modelled as state and sex dependent (St + Sx), whilst survival was modelled as age and sex dependent (a2 + Sx) based on results from initial models of survival and recapture effects (with the base model shown in bold; see Lachish *et al.* 2011 for results of recapture and survival rate modelling)

| Modelling step | Model[a] | $K$[b] | Deviance | QAICc | ΔQAICc | $w_i$[c] |
|---|---|---|---|---|---|---|
| (a) Temporal and environment effects | St*t + St*river[d] | 32 | 11 183·38 | 7520·05 | 0 | 0·793 |
| | St*t + $Inf_{river}$ | 31 | 11 190·94 | 7523·07 | 3·019 | 0·176 |
| | St*t + St*d2river | 32 | 11 200·79 | 7526·548 | 6·489 | 0·031 |
| | $Inf_t$ + $Rec_c$ | 23 | 11 232·89 | 7534·84 | 14·781 | 0·000 |
| | St*t | 30 | 11 212·32 | 7535·29 | 15·236 | 0·000 |
| | St*t + river | 31 | 11 210·65 | 7536·21 | 16·151 | 0·000 |
| | St*river + t | 25 | 11 212·80 | 7539·67 | 19·614 | 0·000 |
| | St*t + d2river | 31 | 11 213·67 | 7547·90 | 27·841 | 0·000 |
| | St*d2river + t | 25 | 11 231·54 | 7547·92 | 27·870 | 0·000 |
| | $Inf_c$ + $Rec_t$ | 23 | 11 231·31 | 7547·95 | 27·896 | 0·000 |
| | $Inf_c$ + $Rec_c$ | 16 | 11 273·67 | 7547·96 | 27·900 | 0·000 |
| (b) Age and sex effects | St*t + St*river | 32 | 11 183·38 | 7520·05 | 0 | 0·190 |
| | St*t + St*river + $Inf_{a2}$ | 33 | 11 180·64 | 7520·26 | 0·200 | 0·171 |
| | St*t + St*river + $Rec_{a2}$ | 33 | 11 181·32 | 7520·71 | 0·656 | 0·136 |
| | St*t + St*river + $Inf_{Sx}$ | 33 | 11 181·92 | 7521·12 | 1·058 | 0·112 |
| | St*t + St*river + $Inf_{a2}$ + $Rec_{a2}$ | 34 | 11 178·94 | 7521·16 | 1·102 | 0·110 |
| | St*t + St*river + $Inf_{a2 + Sx}$ | 34 | 11 179·22 | 7521·35 | 1·287 | 0·100 |
| | St*t + St*river + $Inf_{a2}$ + $Rec_{Sx}$ | 34 | 11 180·50 | 7522·20 | 2·142 | 0·065 |
| | St*t + St*river + $Inf_{Sx}$ + $Rec_{Sx}$ | 34 | 11 190·94 | 7523·07 | 3·012 | 0·042 |
| | St*t + St*river + $Inf_{a2 + Sx}$ + $Rec_{a2 + Sx}$ | 36 | 11 181·87 | 7523·11 | 3·055 | 0·041 |
| | **St*t + a2 + Sx** | **32** | **11 204·02** | **7525·71** | **5·652** | **0·011** |

[a]Model notation described in Table 1. * indicates interaction between variables; + indicates additive effects; covariates in subscripts pertain only to the transition denoted; other covariates pertain to all transitions.
[b]Number of parameters.
[c]Model weight.
[d]Most parsimonious model from step 1 retained for modelling age and sex effects in step 2.

spatially within the study site (Table 2a). Models in which distance to the river was included as an individual covariate were not well supported by the data (ΔQAICc = 6·49 between the best-supported model and the highest ranked model with river as an individual covariate). Estimates from the best-supported model in this stage of the analysis (with 79% of the weight in the candidate set) show that infection rates were on average 55% higher and recovery rates 57% lower in areas located near the River Thames (Fig. 1). This figure also demonstrates the temporal variability in overall infection and recovery rates within the population, with infection and recovery negatively correlated and alternating between higher and lower rates in consecutive years (Fig. 1). The results of model selection examining the effects of host age and sex on infection dynamics revealed that models in which infection and recovery rates varied with host age or sex were amongst the top models but were not better supported than the model with only time dependence and variation in relation to distance to the river (Table 2b). As there was very little difference in the degree of support for the top models in this candidate set, the relative influence of these biotic and abiotic factors on either infection or recovery rates could not be determined from this analysis.

In the species-specific analysis, model selection again revealed very strong support for models in which transition rates varied in relation to the distance from the river (Table 3a), highlighting the importance of this landscape feature for driving variation in the disease incidence rate in the population and influencing the risk of infection for individuals. However, in this analysis, proximity to the river was found to influence only those transition rates which involved C-Clade (*P. circumflexum*) infections: C-Clade infection rate,

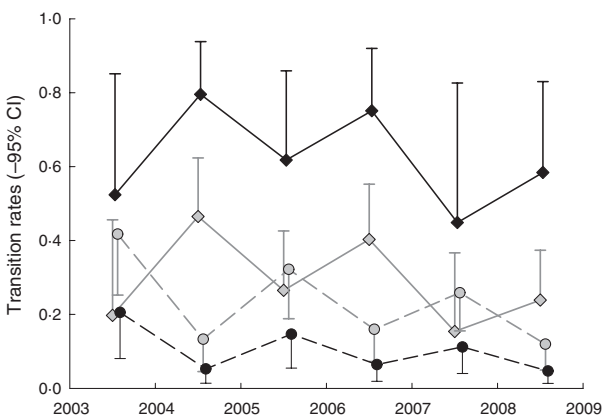


**Fig. 1.** Estimates (±95% CI) from the best-supported model in step 1 of the combined *Plasmodium* multievent mark–recapture analysis showing transition rates of blue tits over the duration of the study in relation to proximity to the River Thames. Infection rates are shown in solid lines; recovery rates are shown in dashed lines; transition rates close to the river shown in black; transition rates far from the river shown in grey. To clarify patterns, not all error bars are shown.

 

**Table 3.** Summary results of multievent mark–recapture analysis modelling transition rates of *Plasmodium relictum* (R-Clade) and *Plasmodium circumflexum* (C-Clade) infections in blue tits. The top 10 models in each modelling step are shown (as well the model with constant transition rates). Recapture rate was modelled as nest box density- and state dependent (St + boxD), whilst survival was modelled as state, time and age dependent (St + t + a2; with the base model shown in bold; see Lachish *et al.* 2011)

| Modelling step | Model[a] | $K$[b] | Deviance | QAICc | ΔQAICc | $w_i$[c] |
|---|---|---|---|---|---|---|
| (a) Temporal and environmental effects | $[RC + CR + CInf + CRec]_{river} + RInf_t + RRec_c$[d] | 40 | 10 562·49 | 7122·64 | 0 | 0·972 |
| | $[RC + CR + CInf + CRec]_{river} + Inf_t + RRec_c$ | 47 | 10 551·98 | 7130·01 | 7·3619 | 0·024 |
| | $[RC + CR + CInf + CRec]_{river} + Inf_t + Rec_t$ | 61 | 10 514·78 | 7134·13 | 11·491 | 0·003 |
| | $[RC + CR + Inf + Rec]_{river} + Inf_t + Rec_t$ | 63 | 10 513·72 | 7137·58 | 14·937 | 0·001 |
| | $[RC + CR + RInf + RRec]_{river} + Inf_t + Rec_t$ | 61 | 10 524·43 | 7140·56 | 17·920 | 0·000 |
| | $[RC + CR + CInf + CRec]_{d2river} + RInf_t + RRec_c$ | 36 | 10 598·24 | 7143·28 | 20·640 | 0·000 |
| | $[RC + CR + CInf + CRec]_{river} + RInf_c + Rec_t$ | 47 | 10 573·86 | 7144·59 | 21·950 | 0·000 |
| | $RC_c + CR_c + Inf_c + Rec_t$ | 43 | 10 587·38 | 7147·32 | 24·681 | 0·000 |
| | $RC_c + CR_c + CInf_c + RInf_t + Rec_c$ | 36 | 10 613·70 | 7148·59 | 25·952 | 0·000 |
| | $RC_c + CR_c + Inf_t + Rec_c$ | 43 | 10 596·69 | 7151·59 | 28·952 | 0·000 |
| | $RC_c + CR_c + Inf_c + Rec_c$ | 29 | 10 655·82 | 7161·88 | 39·240 | 0·000 |
| (b) Age and sex effects | $[RC + CR + CInf + CRec]_{river} + RInf_{t+a2} + CInf_{Sx} + RRec_c$ | 42 | 10 548·45 | 7117·38 | 0 | 0·465 |
| | $[RC + CR + CInf + CRec]_{river} + RInf_{t+a2} + RRec_c$ | 41 | 10 555·40 | 7119·96 | 2·580 | 0·128 |
| | $[RC + CR + CInf + CRec]_{river} + RInf_{t\ +\ Sx} + CInf_{Sx} + RRec_c$ | 42 | 10 553·26 | 7120·58 | 3·208 | 0·094 |
| | $[RC + CR + CInf + CRec]_{river} + RInf_{t+a2} + Rec_{Sx}$ | 42 | 10 555·10 | 7121·81 | 4·435 | 0·051 |
| | $[RC + CR + CInf + CRec]_{river} + RInf_{t+a2+Sx} + RRec_c$ | 42 | 10 555·20 | 7121·88 | 4·503 | 0·049 |
| | $[RC + CR + CInf + CRec]_{river} + RInf_t + Inf_{a2} + RRec_c$ | 41 | 10 558·35 | 7121·93 | 4·549 | 0·048 |
| | $[RC + CR + CInf + CRec]_{river} + RInf_{t+} + Inf_{a2} + Rec_{Sx}$ | 42 | 10 555·86 | 7122·32 | 4·942 | 0·039 |
| | $[RC + CR + CInf + CRec]_{river} + RInf_t + Inf_{a2+Sx} + RRec_c$ | 42 | 10 556·24 | 7122·57 | 5·190 | 0·035 |
| | $[RC + CR + CInf + CRec]_{river} + RInf_t + Inf_{a2} + Rec_{a2}$ | 42 | 10 556·36 | 7122·66 | 5·274 | 0·033 |
| | $[RC + CR + CInf + CRec]_{river} + RInf_t + Rec_{a2}$ | 41 | 10 560·04 | 7123·06 | 5·674 | 0·027 |
| | $\mathbf{RC_c + CR_c + Inf_t + Rec_t + Inf_{a2+Sx} + Rec_{a2\ +\ Sx}}$ | **57** | **10 562·61** | **7141·21** | **4·108** | **0·000** |

[a]Model notation described in Table 1.* indicates interaction between variables; + indicates additive effects; covariates in subscripts pertain only to the transitions denoted (and to all transition rates listed within square brackets).
[b]Number of parameters.
[c]Model weight.
[d]Most parsimonious model from step 1 retained for modelling age and sex effects in step 2.

C-Clade recovery rate and transitions between C-Clade and R-Clade (Table 3a). Models in which R-Clade (*P. relictum*) infection or recovery rates varied with proximity to the river received little support by the data. Again in this analysis, models with distance to the river included as an individual covariate were not well supported by the data (Table 3a). Estimates from the best-supported model at this stage of the analysis (with 97% of the weight in the candidate set) show that C-Clade infection rates were on average 75% greater near the river than further away, whilst recovery rates from C-Clade infections were on average 55% lower near the river than further away (Fig. 2). This model also revealed that transitions from R-Clade to C-Clade infections occurred almost exclusively near the river, whereas transitions from C-Clade to R-Clade infections occurred more often amongst individuals located far from the river (Fig. 2).

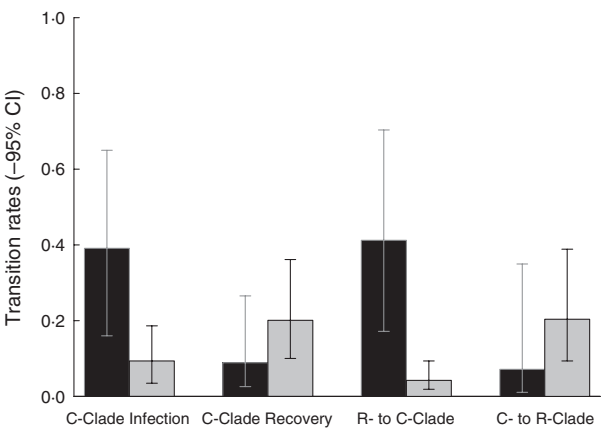


**Fig. 2.** Estimates (±95% CI) from the best-supported model in step 1 of the species-specific multievent mark–recapture analysis showing transition rates of blue tits infected with *Plasmodium circumflexum* (C-Clade) as well as transition rates between *Plasmodium relictum* (R-Clade) and *P. circumflexum* (C-Clade) infections in relation to proximity to the River Thames (transition rates close to the river shown in black; transition rates far from the river shown in grey).

Examining infection dynamics separately for the two malaria species also proved valuable for elucidating patterns of temporal variability in transition rates. In the separate-species analysis, the best-supported model in the first stage of analysis included temporal variation only for R-Clade infection rates (Table 3a). R-Clade infection rates alternated between higher and lower values in consecutive years (Fig. 3a), suggesting that the temporal variation observed in the overall disease incidence rates in the population is largely driven by temporal variation in R-Clade infection rates.

Incorporating species information on infection status likewise afforded us a clearer understanding of the effects of host age and sex on infection dynamics. In this analysis, model selection revealed substantial support for models in which infection rates, but not recovery rates, varied with host age or

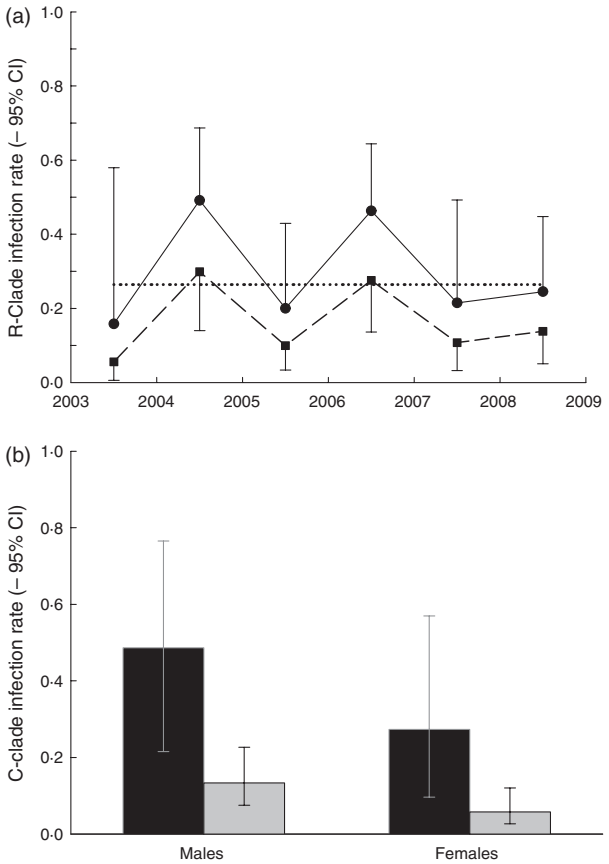


**Fig. 3.** Results of species-specific mark–recapture analysis showing (a) the effect of age (circles and solid lines for yearlings; squares and dashed lines for adults) on the infection rates of blue tits with *Plasmodium relictum* (R-Clade), as well as the recovery rate from R-Clade infections for blue tits (shown as dotted line); and (b) the effect of sex on infection rates of blue tits with *Plasmodium circumflexum* (C-Clade) as a function of proximity to the river (close to the river shown in black; far from the river shown in grey). Estimates are model-averaged means (±95% CI). To clarify patterns, not all error bars are shown.

sex (Table 3b), suggesting that these biotic traits influence the rate at which individuals become infected, but not the rate at which they clear infections. Furthermore, the model selection process revealed that the effects of these biological traits on infection rates differed between the two malaria species. The best-supported model in the candidate set showed that C-Clade infection rates were influenced by host sex alone, whilst R-Clade infection rates were influenced only by host age (Table 3b). Model-averaged estimates show that R-Clade infection rates were higher for adults than for yearlings (Fig. 3a), whilst C-Clade infection rates were higher for males than for females, regardless of proximity to the river (Fig. 3b).

## Discussion

Using a novel multievent mark–recapture framework (Choquet 2009; Conn & Cooch 2009) to account for infection state–dependent differences in host detectability, we found evidence of striking spatial heterogeneity in malaria transmission rates within a single population of blue tits over a small spatial scale. Moreover, we found marked differences in the role of environmental and host factors in forcing infection dynamics of the different malaria species within this population and also documented distinct patterns of temporal variation in infection dynamics of these two malaria species. Thus, this study documents that the processes causing infection by pathogens in natural populations are far from homogenous in space or time and also demonstrates that knowledge of the drivers of spatial and temporal heterogeneity in disease transmission will be crucial for developing accurate epidemiological models and a thorough understanding of the evolutionary implications of pathogens. The scale over which these variable effects were demonstrated is rather small; individuals living a few hundred metres apart (which is considerably less than the median natal dispersal distance in this population; S. C. L. Knowles & B. C. Sheldon, unpublished) were subject to quite different forces of infection as well exposure to potentially different pathogens.

Abiotic factors have been shown to play a particularly prominent role in driving the transmission dynamics of vector-borne pathogens (Randolph 2001; Byers *et al*. 2008; Borer *et al*. 2010), including malaria (Foley *et al*. 2003; Balls *et al*. 2004). Environmental drivers of malaria transmission include altitude, climatic conditions and proximity to permanent water, factors that can either limit the abundance and distribution of vectors or impinge on parasite vigour (Foley *et al*. 2003; Balls *et al*. 2004; Freed *et al*. 2005; Pope *et al*. 2005; Atkinson & LaPointe 2009). In this study, we found that proximity to the River Thames, the only large permanent water source in or near to our study site, influenced the transmission rates of *P. circumflexum*, but not of *P. relictum*. Our results revealed that this was not a monotonic linear effect with increasing distance from the river but better described as a dichotomous effect of proximity to permanent water *per se*. Indeed, the disease incidence rate of *P. circumflexum* was nearly four times greater and consequently, the likelihood of recovery substantially lower in areas within 500 m of the river than in areas further away (explaining the similar pattern observed for combined *Plasmodium* transmission rates). This result clearly indicates that proximity to the river is a key determinant of host infection risk for *P. circumflexum* in this population. This result is consistent with the expectation that *P. circumflexum* and *P. relictum* are transmitted by different vectors, with the mosquito species responsible for transmitting *P. circumflexum* being restricted within the study site by the availability of wet larval habitat for breeding. Preliminary investigations into mosquito ecology at our study site have found a total of 14 mosquito species, or species complex, with overall mosquito abundance varying spatially through the study site (R. Alves, M. J. Wood, C. Cowell, B. C. Sheldon, unpublished data). The most abundant species in the area near the River Thames, *Ochlerotatus annulipes* (R. Alves, unpublished data), lays its eggs exclusively on damp soil or leaf litter (Cranston *et al*. 1987) and is a potential candidate vector for *P. circumflexum*

transmission. Clearly, further investigation into the biology and ecology of vectors within our study site, and particularly their vector competency with respect to the different avian malaria species, is needed to be able to verify this suggestion.

Infection rates of *P. relictum* amongst birds, although not influenced by proximity to the River Thames, did display marked variation between years in this study and were the primary driver of similar temporal variation observed in overall disease incidence rates of *Plasmodium* in the population. Although these two processes need not be correlated, the observed pattern of variation in infection and recovery rates between years may be explained solely by changes in the underlying force of infection in the population. A high force of infection will result in many new infections in hosts, but few observed recoveries, whilst more recoveries and fewer new infections will be observed when the force of infection is low. One potential explanation for this marked annual variation in the underlying force of infection in the population is that the vector or vectors responsible for *P. relictum* transmission fluctuate in abundance according to annual climatic variation (e.g. temperature and rainfall), which alter the microhabitat or microclimates they require for breeding. Greater transmission rates may thus occur in years when conditions are more favourable for vectors. Alternatively, annual variation in host demography and population dynamics could also play a role in driving this temporal variability, via periods of greater or lower immigration or recruitment of immunologically naive individuals (Anderson & May 1986; Atkinson & Samuel 2010). However, the effects of annual variation in host demography and population dynamics would presumably also be expected to affect infection dynamics of other *Plasmodium* species, and very little temporal variation was observed for *P. circumflexum* transmission. Again, an understanding of the life cycles and behaviour of the mosquito species in our study system and their vector competency would assist in explaining the observed pattern of temporal variation in disease incidence rates and of *P. relictum*.

Although host factors such as age and sex are known to predict the prevalence of malaria infection in a variety of avian hosts, although not always in a consistent manner (Korpimäki, Hakkarainen & Bennett 1993; Marzal *et al*. 2008; van Oers *et al*. 2010), few ecological studies have yet explored their influence on transmission in the wild (Atkinson & Samuel 2010). In this study, we found that malaria infection rates, but not recovery rates, were influenced by host age and sex, but that the manner in which these biotic factors influence transmission differed between the two *Plasmodium* species. Infection rates for *P. circumflexum* were greater for males than for females. This pattern might result from differences in life history (e.g. if males settle earlier on breeding territories and are thus exposed for longer) or reproduction or foraging behaviours (e.g. males do not incubate or may spend more time foraging) causing male blue tits to experience greater exposure to vectors of this parasite species. Such differences in life history may not have resulted in detectable differences in *P. relictum* infection rates for males and females, because *P. relictum* is more patchily distributed within the study site than *P. circumflexum* (Wood *et al*. 2007). More detailed analysis of individual variation in settlement time or foraging behaviour might shed light on these hypotheses. Infection rates for *P. relictum* did vary between yearlings and adults, however, with adults being more likely to acquire infection over a yearly interval. Whilst it is possible that a difference in the length of exposure for adults and yearlings could affect the rate at which they acquire infections, it is difficult to understand why this would only manifest in an effect for *P. relictum* and not *P. circumflexum*. In addition, this pattern cannot be explained by greater survival prospects for infected adults, as the previous work has shown that infected adults have lower survival rates than infected first years (Lachish *et al*. 2011). Although we can only speculate at present, age-specific differences in immune function (as it relates to the likelihood of infection relapse) could play a role in driving these differences in transmission rates (Wood *et al*. 2007; van Oers *et al*. 2010). Clearly, further work is needed to clarify the physiological and ecological mechanisms by which age and sex influence transmission rates of different *Plasmodium* species.

One limitation of the assessment of the role of biotic and abiotic factors in driving malaria infection dynamics in this study is that infection status was only tested annually during the host's breeding seasons. The timing of malaria transmission in this population is not precisely known, but preliminary data suggest that the majority of infections may be acquired after the breeding season in this population, in mid to late summer (when vectors are expected to be most abundant and also when immunologically naive juvenile enter the host population), with negligible transmission assumed in winter (as vector activity wanes and parasites disappear from the blood; Cosgrove *et al*. 2008). Thus, not only are we likely to have missed many transitions amongst infection states, but our transition rate estimates clearly reflect the combined effect of these varied seasonal dynamics on infection and recovery processes. Further investigation into the drivers of transmission dynamics would help to clarify to what extent infections represent new infections or relapses of previous infections, and to what extent host vs. environmental factors drive recovery (infection loss) rates outside of the winter period. However, as the seasonal pattern of malaria transmission still needs to be characterized, there remain significant gaps in our understanding of the infection dynamics of avian malaria in natural populations.

Another challenge in relating estimates of transition rates obtained in this analysis to true infection rates in the population is that our previous work has shown that disease-induced mortality differs between the two parasite species (Lachish *et al*. 2011). Differences in disease-induced mortality of hosts will cause transition rate estimates to be biased to differing degrees for each of the *Plasmodium* species. Infection rates for *P. circumflexum* (which substantially reduced host survival relative to *P. relictum*) will have been underestimated in this study, whereas infection rates for *P. relictum* will have been estimated with little bias, as the negative

impacts of *P. relictum* infection on host reproductive output seemed, if anything, to benefit (improve) host survival prospects (Lachish *et al.* 2011). Nevertheless, as even biased transition rates still provide a conservative (under) estimate of underlying variation in disease transmission, they can still contribute to inference on infection dynamics in this system, though care is needed in their interpretation, as the magnitude of the relative rates may not reflect reality.

One further caveat to the inferences drawn in this study is that the multievent mark–recapture model, like most standard multistate analyses, assumes that state transitions are first-order Markovian, such that the probability of an individual making a transition between time $i$ and $i + 1$ depends only on its state at time $i$ (Williams, Conroy & Nichols 2001; Pradel 2005). The possibility of relapses of prior infections, as well as differences in host-acquired immunity to infection, implies that this may be a naive assumption for this disease system. Extensions of multievent models allow state transitions to be modelled as higher-order Markovian process (so-called memory models, as per Hestbeck, Nichols & Malecki 1991). Although these memory models will be critical for elucidating the role of host immune response and host genetic factors in the transmission dynamics of diseases like avian malaria, they demand vast amounts of data and are thus difficult to implement in reality. Certainly, the sparseness of the relevant aspects of our data (over 9 years only 175 transitions between known disease states in 4843 capture of 3424 individuals) prevented us from modelling state transitions as a higher-order Markov process.

Investigating temporal and spatial patterns of infection dynamics in wild populations can inform about the role of environmental factors in mediating host–pathogen interactions. In this study, we documented striking spatial variation in the transmission dynamics of two avian malaria species within a single population of blue tits. This indicates that landscape variables are capable of driving avian malaria transmission at much smaller spatial scales than previously recognized (Perez-Tris & Bensch 2005; Atkinson & Samuel 2010; Loiseau *et al.* 2010). The fact that these differences in transmission intensity occur at a local scale (over a few hundred metres) also suggests that the selective effects of malaria infection on avian hosts can be very different over very small spatial scales. Consistent differential selection pressures for different genetic aspects of host resistance in spatially segregated areas can lead to local adaptation of hosts and parasites (Dybdahl & Storfer 2003). Although spatial variation in the disease incidence rate of *P. circumflexum* in this population was shown to be stable over the study, dispersal and immigration rates are high in the population, diminishing the potential for host–parasite co-evolution. Nonetheless, if dispersal is non-random with respect to resistance phenotype, then local adaptation might occur even in the face of marked dispersal (see Garant *et al.* 2005 for an example of this process in a different context). On the other hand, because proximity to the River Thames was influential for determining overall risk of malaria infection in this population, and infection carries substantial fitness costs for hosts (Lachish *et al.* 2011), the potential for host dispersal to mediate the impacts of infection for hosts is evident. Further investigation as to whether differential dispersal in juveniles and adults can reduce subsequent parasitism rates and lead to optimal dispersal strategies for disease avoidance in this system is clearly warranted. Overall our findings demonstrating that the role of environmental and host factors in driving patterns of avian malaria transmission can vary dramatically between different *Plasmodium* species suggest that future attempts to understand the epidemiology, ecology and evolutionary implications of avian malaria parasites in wild populations should account not only for temporal and spatial variation in factors affecting vector distribution and abundance and host demography, but also the diversity of haematozoan species present within the host population.

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## Supporting Information

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

**Appendix S1.** M-arrays providing a concise summary of the mark–recapture datasets used in multi-event modelling of the infection dynamics of (a) combined *Plasmodium* infection in wild blue tits; and (b) species-specific *Plasmodium* infection dynamics.

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