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Fitness effects of endemic malaria infections in a wild bird population: the importance of ecological structure

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Summary

1. Parasites can have important effects on host populations influencing either fecundity or mortality, but understanding the magnitude of these effects in endemic host–parasite systems is challenging and requires an understanding of ecological processes affecting both host and parasite.
2. Avian blood parasites (*Haemoproteus* and *Plasmodium*) have been much studied, but the effects of these parasites on hosts in areas where they are endemic remains poorly known.
3. We used a multistate modelling framework to explore the effects of chronic infection with *Plasmodium* on survival and recapture probability in a large data set of breeding blue tits, involving 3424 individuals and 3118 infection diagnoses over nine years.
4. We reveal strong associations between chronic malaria infection and both recapture and survival, effects that are dependent on the clade of parasite, on host traits and on the local risk of infection.
5. Infection with *Plasmodium relictum* was associated with reduced recapture probability and increased survival, compared to *P. circumflexum*, suggesting that these parasites have differing virulence and cause different types of selection on this host.
6. Our results suggest a large potential survival cost of acute infections revealed by modelling host survival as a function of the local risk of infection.
7. Our analyses suggest not only that endemic avian malaria may have multiple fitness effects on their hosts and that these effects are species dependent, but also that adding ecological structure (in this case parasite species and spatial variation in disease occurrence) to analyses of host–parasite interactions is an important step in understanding the ecology and evolution of these systems.

Key-words: avian malaria; plasmodium, blue tits (Cyanistes caeruleus), host–parasite interactions, life-history trade-offs, multistate mark–recapture models

Introduction

Parasites that reduce the fitness of their hosts will constitute a strong selective force in natural populations and have the potential to exert important evolutionary and ecological pressures in the wild (Poulin 2007). It is now widely acknowledged that the negative impacts of parasites on host fitness can play an important role in regulating host populations (Albon *et al*. 2002), in driving host population cycles (Hudson, Dobson & Newborn 1998) and, in the extreme, can even limit population persistence and viability (McCallum *et al*. 2009; Smith, Acevedo-Whitehouse & Pedersen 2009). In addition to potential effects on population dynamics, the manner in which parasites reduce host fitness can also influence host life-history evolution (Sheldon & Verhulst 1996), especially because host immune defences and behavioural traits may evolve in different ways in response to impacts on fecundity as opposed to survival, or to impacts incurred at different life-history stages (Lochmiller & Deerenberg 2000; Moller, Martin-Vivaldi & Soler 2004). Hence, to understand the ecological and evolutionary implications of parasites on their hosts, it is essential to quantify host–parasite interactions, particularly the effects of parasites on hosts in the wild.

Malaria parasites (e.g. *Haemoproteus*, *Plasmodium*) and their avian hosts have frequently been used as a model system to investigate host–parasite interactions, co-evolutionary processes and the role of parasites in host life-history evolution (Galvani 2003; Ricklefs, Fallon & Bermingham 2004; Ricklefs & Outlaw 2010). Nevertheless, despite much research, the impacts of blood parasites on host fitness and

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host population dynamics in the wild remain poorly understood. In naive host populations, malaria parasites have sometimes been shown to dramatically increase host mortality, reduce population abundance and limit species distributions (van Riper *et al*. 1986; Atkinson & van Riper 1991; Atkinson & Samuel 2010). They have also been shown to have detrimental impacts on host survival in captive and domestic birds (Atkinson & van Riper 1991; Williams 2005; Palinauskas *et al*. 2008). However, in wild populations where malaria transmission is endemic and hosts have a long evolutionary history with these parasites, studies provide conflicting results as to whether such infections have appreciable fitness effects for hosts (e.g. Korpimäki, Hakkarainen & Bennett 1993; Sanz *et al*. 2001; Marzal *et al*. 2005; Bensch *et al*. 2007).

There are several reasons why detecting the impacts of malaria parasites on host fitness in endemic areas is difficult. As the acute stage of malaria infection (when parasitaemia is high) is very brief and can entail significant mortality costs, the vast majority of infected individuals in natural populations will be 'survivors', which as a result of host-acquired immunity harbour only chronic infections (Valkiūnas 2005; Atkinson & Samuel 2010). Hosts with chronic infections show greatly reduced parasitaemia levels and may bear minimal fitness costs of infection (Valkiūnas 2005; Bensch *et al*. 2007). Moreover, if the impacts of acute infections occur over short time scales and the impacts of chronic infections are small, then in the absence of direct experimentation (e.g. Knowles, Palinauskas & Sheldon 2010a) detecting effects on hosts will require both large sample sizes and long-term data on the traits of infected and uninfected individuals in endemic populations (McCallum & Dobson 1995). Such longitudinal studies of the infection dynamics of avian malaria in wild populations where transmission is endemic remain rare.

The assessment of fitness costs of malaria infection in the wild is also complicated by the considerable diversity of malaria species that may comprise infections in host populations (Bensch *et al*. 2004; Waldenström *et al*. 2004). Pathogen virulence may vary among species, and parasite species may also differ in the nature of their impacts on hosts (Lively 2006; Palinauskas *et al*. 2008). Hence, the presence of multiple, cryptic or unrecognized malaria species within a host population can potentially obscure any fitness effects. To date, few studies have explicitly considered the possibility that infection dynamics and host–parasite interactions may vary with malaria species (Wood *et al*. 2007; Marzal *et al*. 2008; Ortego *et al*. 2008).

Another important, but often overlooked, problem when attempting to assess disease impacts in the wild is the issue of detectability or capture heterogeneity (Jennelle *et al*. 2007). Pathogen-induced changes in behavioural traits, activity levels or other physiological processes can lead to significant heterogeneity in the probability of sampling infected and uninfected individuals (Senar & Conroy 2004). If disease-dependent variation in capture probabilities exists but is ignored, then observed patterns (e.g. prevalence or survival rates) and any inference based on them may simply be artefacts of host encounter rates or conceal important biologically relevant effects because of biases (Jennelle *et al*. 2007). The majority of ecological studies of host–parasite interactions in avian malaria systems have not considered issues of state-dependent detectability. This is particularly worrying in populations infected with multiple malaria species, as it is possible that hosts infected with different species are encountered at different rates, particularly if there is spatial variation in the distribution of those species (e.g. Wood *et al*. 2007). Multistate mark–recapture models (MSMR) provide a framework for assessing disease impacts in wild populations, while explicitly accounting for variability in detection rates with infection status (Conn & Cooch 2009). Although fast becoming an integral tool in wildlife disease ecology, such models have rarely been used to assess fitness consequences in avian malaria systems (Vanderwerf 2008; Atkinson & Samuel 2010).

In this study, we utilized MSMR models to assess the fitness consequences of malaria in a long-term monitored population of blue tits (*Cyanistes caeruleus*), infected with two divergent *Plasmodium* parasite species (*P. relictum* and *P. circumflexum*, Valkiūnas 2005). We aimed to determine whether these malaria parasites have significant fitness effects for hosts when transmission is endemic and in particular the extent to which impacts of infection differ with respect to the *Plasmodium* species infecting individuals and as a function of host traits (age and sex).

Materials and methods

STUDY SITE AND HOST SPECIES

Blue tits are small passerine birds that take readily to nestboxes. In the UK, blue tits are resident year-round and lay eggs in spring with the peak of broods hatching (in southern England) from late April to early May. From 2001 to 2009, 250–450 pairs of individually marked blue tits were monitored in Wytham Woods (51°46′N, 1°20′W), near Oxford, UK. The 385-ha study site is a continuous mixed semi-deciduous forest (complete description of study area in Perrins 1979), in which approximately 1160 nestboxes are distributed at variable densities. Blood samples for infection diagnosis were collected annually from breeding blue tits captured between day 6 and 14 of the nestling phase, either within the nestbox by hand or using traps, or with mist nets in front of the nest entrance. As the study population is single brooded and breeding is highly synchronous, there is little variation in the calendar date among samples within each year (average range ± SE = 42·42 ± 6·78 days). However, the proportion of all captured birds from which blood samples were obtained (and analysed) did vary across years (Table 1). Host sex was determined based on the presence (female) or absence (male) of a brood patch, while age (yearling or adult) was determined using plumage characteristics (Svensson 1992) or ringing records for birds ringed as nestlings. As an exact age could not be assigned for a significant proportion (23%) of captured adults, we restricted age effects in survival analyses to two age classes: yearlings (1 year olds) and adults (2+ year olds).

AVIAN MALARIA PARASITES AND MOLECULAR DIAGNOSIS OF INFECTION

Previous molecular characterization of haemosporidian infections in this population has shown that infections of two well-defined

Table 1. Total number of individuals (and of each sex) captured in each year of the study and the number of these for which diagnoses were and were not obtained (by disease state and by parasite species)

Year	No. captured (No. F/M)	No. tested for malaria	Molecular method	No. State unknown*	No. species unknown†	Apparent prevalence for species combined (±95% CI)	Apparent/corrected prevalence‡ *Plasmodium relictum*	*P. circumflexum*
2001	429 (230/199)	182	Nested PCR	247	1	0·151 (0·110, 0·224)	0·066/0·088	0·088/0·069
2002	517 (283/234)	0	NA	517	NA	NA	NA	NA
2003	572 (318/254)	48	Nested PCR	524	0	0·101 (0·034, 0·227)	0·063/0·082	0·042/0·032
2004	473 (244/229)	398	Nested PCR	75	9	0·302 (0·257, 0·349)	0·138/0·187	0·138/0·109
2005	494 (273/221)	472	Nested PCR & qPCR	66	32	0·521 (0·475, 0·567)	0·248/0·339	0·214/0·171
2006	475 (273/202)	472	Nested PCR & qPCR	3	17	0·445 (0·399, 0·491)	0·218/0·287	0·210/0·161
2007	523 (304/219)	517	Nested PCR & qPCR	6	25	0·484 (0·440, 0·528)	0·310/0·386	0·149/0·109
2008	525 (284/241)	523	qPCR	2	7	0·363 (0·322, 0·406)	0·136/0·184	0·214/0·169
2009	509 (259/250)	506	qPCR	3	7	0·389 (0·347, 0·433)	0·121/0·167	0·251/0·203

Also shown are apparent prevalence estimates (number infected/total individuals captured) for malaria species combined and both apparent and corrected prevalence estimates for each species separately.

*State was unknown for individuals that were not tested for *Plasmodium* infections.

†Species was unknown for individuals not tested for *Plasmodium* infection, for mixed infections (infections with both *Plasmodium* Species), and for infections in which the parasite species could not be ascertained.

‡Prevalence estimates were corrected for state-dependent recapture rates using mean state-dependent recapture rates from model p(St + boxD) (see Table 4) according to the following formula:

$$\mathrm{Prev}_{\mathrm{corr}} = \frac{\frac{C_{\mathrm{cladeA}}}{p_{\mathrm{cladeA}}}}{\left(\frac{C_{\mathrm{cladeA}}}{p_{\mathrm{cladeA}}} + \frac{C_{\mathrm{cladeB}}}{p_{\mathrm{cladeB}}} + \frac{C_{\mathrm{Uninf}}}{p_{\mathrm{uninf}}}\right)}$$

where C is the count of individuals in different states and p is the recapture rate for that state.

Plasmodium morphospecies (based on cytochrome *b* sequences), *P. relictum* and *P. circumflexum* (Palinauskas *et al.* 2007) are common during the breeding season and constitute >98% of blood parasites in the genera *Plasmodium* and *Haemoproteus* (Knowles *et al.* 2011). We refer to these parasites by their morphospecies classification, but to simplify model notation (see CMR results below) we refer to *P. relictum* as 'R-Clade' and *P. circumflexum as* 'C-Clade'. Although 'morphospecies' classifications can conceal much greater species diversity at a finer level of molecular resolution (Bensch *et al.* 2004), in this population both clades consisted of predominantly one parasite lineage (cytochrome *b* sequence, see Table S1). Moreover, phylogenetic analyses revealed far greater divergence between the two clades than among any of the lineages within the clades (Wood *et al.* 2007). For these reasons, and also because the multistate models are very data intensive, we restrict our comparisons to these two historically divergent morphospecies ('species' for simplicity).

Samples were screened for parasites either by nested polymerase chain reaction (PCR) assays from 2001 to 2004 (protocols described in Wood *et al.* 2007; Waldenström *et al.* 2004 and) or quantitative (q)PCR assays from 2005 to 2009 (protocols details described in Knowles *et al.* 2011). DNA quantification during qPCR assays revealed that, as expected, the majority of infected hosts harboured chronic infections with very low parasitaemia loads (>90% of infected hosts possessed parasitaemia values two orders of magnitude lower than the maximum values recorded, unpub. data).

A proportion of samples were screened by both methods (30%, Table 1) and revealed a high degree of concordance both in the diagnoses given by the two methods (78%; $n = 1042$) and in the malaria species assigned to infected diagnoses (96%, $n = 302$; Knowles *et al.* 2011). However, because qPCR assays involved analyses of samples in triplicate (with a positive diagnosis if at least one replicate was positive), whereas nested PCR assays involved a single analysis of each sample, the overall detection rate for qPCR was greater than for nested PCR (82% of mismatches involved a positive diagnosis by qPCR and a negative diagnosis by nested PCR). Where diagnoses differed, individuals were considered uninfected only if both methods gave uninfected diagnoses. If either method gave an infected diagnosis, then individuals were considered to be infected. Hence, we assume that false positives by either method are negligible. This was justified because strict laboratory protocols minimized false positives: results were only used when all negative controls on a PCR plate showed no contamination; samples that returned equivocal results were retested and if still ambiguous then they were designated as 'unknown' in model analyses.

Although false-positive diagnoses will be rare in this study, false-negative diagnoses may have occurred. If not accounted for, false-negative diagnoses will introduce negative bias in survival estimates. A recent simulation study has revealed, however, that 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 there will be very little bias in estimates (McClintock *et al.* 2010). Because the majority of diagnoses in this study were undertaken with qPCR in which samples are analysed in triplicate, and because only two survival estimates were obtained in the years in which nested PCR was used (see Results), we believe that the potential for biased survival estimates to produce spurious inferences in this study is small. Moreover, exclusion of years 2001–2003 from analyses did not qualitatively change the results of recapture and survival rate modelling presented below.

Mixed species infections were rare in this population (<5% of all infections) and were treated as 'infected' in the combined-species analysis and as 'unknown' in the species-specific analysis, as were the few occasions ($N = 46$) where there was disagreement between the two molecular methods in the *Plasmodium* species diagnosed (see Knowles *et al.* 2011 for species diagnosis methods).

MSMR MODELLING APPROACH

The mark–recapture data set consisted of yearly capture histories for all breeding birds captured within the study site from 2001 to 2009,

grouped by sex and age and assigned to different disease states according to their infection status at the time of capture. Because not all individuals were tested for malaria in all years, the infection status of a proportion of birds was not known (Table 1). To accommodate these undiagnosed individuals within our MSMR framework, we employed newly developed 'multievent' models, which explicitly account for unknown or partially observable states by treating them as a hidden Markov process (Pradel 2005; Conn & Cooch 2009). We employ the general model structure described in Conn & Cooch (2009), which allows for both the detection process and the process of obtaining data on infection status conditional on being detected to be modelled (see Fig. 2 in Conn & Cooch 2009). Incorporating unknown disease states directly into the estimation process increases the precision of parameter estimates and is a significant improvement over the alternative options of either censoring such individuals or assigning them to a separate state (Faustino *et al.* 2004; Conn & Cooch 2009).

We conducted two multievent mark–recapture analyses to assess the impacts of endemic malaria in our study population and demonstrate the importance of accounting for parasite species. In the first analysis, we combined both *Plasmodium* species into a single infected state to examine the overall impact of endemic malaria infection on host survival and recapture. The data set for this analysis included all captured individuals, regardless of whether information on their infection status was known. Capture histories 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, see Fig 1a; the 'dead' state is explicitly included in all multievent models, Pradel 2005). In the second analysis, we kept both *Plasmodium* species separate and examined species-specific impacts. In this analysis, capture histories were assigned 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 both analyses, transitions between all states were possible. Here, we report the results of survival and recapture rate modelling; a further paper (Lachish *et al. submitted*) will report the results of transition rate modelling and assess patterns of infection and recovery rates within the population.

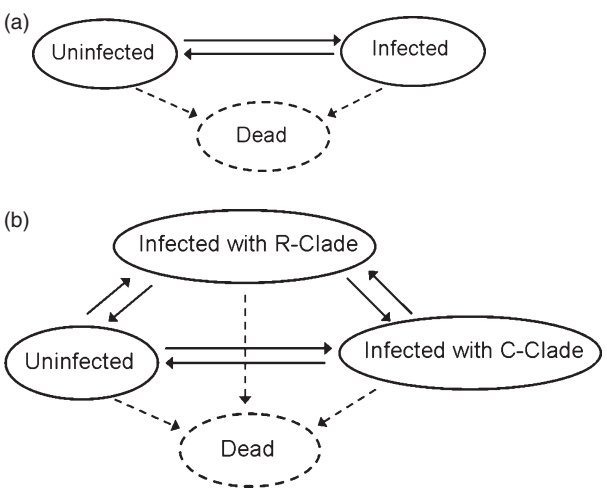

Fig. 1. Multievent mark–recapture model structure for (a) combined *Plasmodium* analysis and (b) species-specific analysis (C-Clade = *P. circumflexum*; R-Clade = *P. relictum*) used for modelling survival and recapture rates of blue tits.

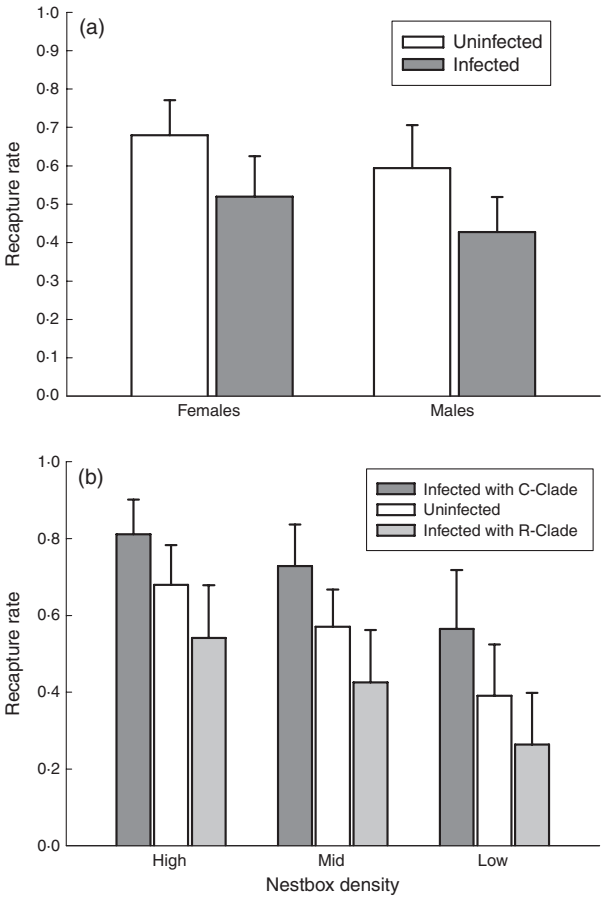

Fig. 2. Results of multievent mark–recapture modelling of recapture rates showing (a) the effect of combined *Plasmodium* infections on recapture rates of uninfected and infected, male and female blue tits; and (b) effect of *P. relictum* (R-Clade) or *P. circumflexum* (C-Clade) infection on the recapture rate of blue tits in areas of high, medium or low nestbox density. Estimates are model averaged means ± 95% CI.

We aimed to quantify differences in recapture and survival rates among infected and uninfected individuals and assess whether effects of infection differed in relation to host factors. Our global models thus included the effects of disease state, time (yearly variation), host sex and host age. Unfortunately, our data were too sparse to fit full time-dependent models for all parameters. In both analyses, the initial capture probability (which estimates the probability of being in a given state when first encountered, Pradel 2005) was time invariant and set to vary with disease state. Also in both analyses, we set the partial observation parameter (which estimates the underlying probability of observing disease state, given that the individual is captured, Conn & Cooch 2009) to vary only by disease state (infected vs. uninfected) and the molecular protocol used for diagnosis (nested PCR vs. qPCR). In addition, in the species-specific analysis, changes in infection status from R-Clade to C-Clade infection or vice versa were modelled as time invariant, as these transitions were extremely sparse in our data set. As goodness-of-fit tests are not currently available for reduced parameter multievent models (Pradel 2005; Choquet *et al.* 2009), we accounted for potential lack of fit in our data in the following ways: (i) by including models with trap dependence in capture probabilities in the candidate model set to account for the possibility of heterogeneity in recapture rates between individuals; (ii) by including models with age effects to account for the possibility of

transience (an excess of newly marked individuals that are never seen again), and (iii) by using a reasonably large variance inflation factor ($\hat{c}$ = 1·5) for conservative model selection (Faustino *et al*. 2004; Choquet *et al*. 2009).

For each analysis, we first modelled variation in recapture rates in relation to disease state, time, trap dependence and sex (preliminary results yielded no evidence that recapture rates varied with age), with survival and transition rates fully parameterized. To assess whether observed differences in clade-specific recapture rates (see Results) were driven by factors that were spatially confounded with the distribution of the two *Plasmodium* species in the population, rather than infection with the parasite per se, we also included models in which recapture rate varied with the density of nestboxes in the woodland area in which individuals were captured or with the distance individuals were from the edge of the woodland (to allow for greater emigration by individuals in the edge). Each of the nine woodland areas in the study site (see Fig. 2 in Wood *et al*. 2007) was classified as having either high (> 7 nestboxes/ha: C, B), medium (3–4 nestboxes/ha: O, P, MP, CP, W) or low (< 2 nestboxes/ha: E, SW) nestbox density. Distance from the edge of the study site was calculated per nestbox using GIS, with each nestbox classified as being either near (within 100 m) or far from the woodland edge.

Next, we modelled variation in survival rates in relation to disease state, time (year), sex and age, using the most parsimonious recapture rate model identified in step one. In addition to models with state-dependent survival rates, we also included models in which host survival rates varied between areas of high and low *Plasmodium* prevalence (preliminary investigations showed this measure of infection risk to be much better supported than measures involving only one or other of the *Plasmodium* species). These models were included to explore the possibility of acute effects of infection on host survival. We reasoned that if purportedly uninfected hosts have lower survival rates in high-prevalence areas, where the force of infection is high, then this would indicate that acute infections carry a fitness cost for hosts (because birds in high-prevalence areas would be more likely to have acquired infection and died soon after infection without this transition appearing in our data set). Average interpolated *Plasmodium* prevalence from 2005 to 2009 (the years when qPCR was used) was calculated per nestbox using inverse distance weighted interpolation (described in Wood *et al*. 2007). We chose 40% prevalence (close to the mean prevalence; Table 1) as a cut-off point to distinguish high-prevalence from low-prevalence sites.

In mark–recapture analyses, survival is confounded with permanent emigration. We assessed whether the observed differences in the apparent survival rates of C-Clade and R-Clade infected individuals (see Results) could be attributed to differences in emigration rates in two ways. First, we included models in the candidate set in which survival rates varied between individuals captured close to and far from the woodland edge, to verify that edge-dwelling individuals were not more likely to 'emigrate' (less likely to survive). Second, we compared the distance moved within the study site (breeding dispersal distances) of uninfected individuals and individuals infected with the two clades as an index of likely emigration rate (assuming that greater within-site movement might predict an increased propensity to emigrate). We also assessed whether the observed survival differences were because of underlying habitat quality by comparing the abundance of oak trees within 50 m of nestboxes (a strong predictor of habitat quality and breeding success, Perrins 1991) in high and low-prevalence areas (Wilkin, Perrins & Sheldon 2007, Wood *et al*. 2007).

All models were fitted to the data using program E-SURGE (Choquet 2009). To ensure convergence of models on the global minima, models were run using repeated random initial values ('multiple random' option with N = 8; Choquet 2007). We investigated the additive and interactive effects of model variables up to two-way interactions between main effects (more complicated models were not well supported). Model selection was based on small sample size corrected Akaike Information Criteria adjusted for overdispersion (QAICc), with models that differed in QAICc values by < 2 considered equivalent in their ability to describe the data (Burnham & Anderson 2002). The relative likelihood of each model in a candidate set was estimated with normalized QAICc *w*eights (w_i, or the index of relative plausibility). We obtained robust parameter estimates through model averaging (Burnham & Anderson 2002) and, where cited, effect sizes of disease state on recapture and apparent survival rates (on the logit scale) were also model averaged from relevant models. Model notation is explained in Table 2.

Table 2. Notation used to denote the main effects and model structure for modelling recapture rate (p) and apparent survival rate (Φ)

Model notation	Description	Parameter
St	State-dependent effect	p, Φ
U	Uninfected individuals	p, Φ
I	Infected individuals (clades combined)	p, Φ
R	Individuals infected with R-Clade (*Plasmodium relictum*)	p, Φ
C	Individuals infected with C-Clade (*P. circumflexum*)	p, Φ
Covariate effects		
Sx	Sex effect	p, Φ
a2	Age effect (yearlings or adults)	Φ
boxD	Density of nestboxes (low/mid/high)	p
edge	Distance from the edge of the woodland (near/far)	p, Φ
prev	Local prevalence of *Plasmodium* (high/low)	Φ
trap	Trap dependence effect	p
t	Time dependence (yearly variation)	p, Φ

Results

A total of 3424 birds were captured an average of 1·4 times for a total of 4843 captures over the nine years of the study (Table 1). We observed 175 transitions between known disease states (the uninfected and infected states) in the combined-species analysis and 167 transitions among 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). In years where > 80% of individuals were tested, the apparent prevalence of *Plasmodium* in the population ranged from 30 to 52%, with the prevalence of *P. relictum* (R-Clade) more variable among years than the prevalence of *P. circumflexum* (C-Clade; Table 1).

RECAPTURE RATES

All the most parsimonious models in the combined-species analysis contained an effect of disease state on recapture rates

Table 3. Summary results of the multievent mark–recapture analysis modelling the effect of combined *Plasmodium* infections on recapture and survival rates of blue tits

Parameter	Model	k	Deviance	QAICc	ΔQAICc	*w*
(a) Recapture rates (p)	St + Sx†	47	11170·91	7533·30	0	0·394
	St	46	11175·71	7535·28	1·982	0·146
	St × Sx	48	11169·69	7535·85	2·546	0·110
	St + trap	47	11172·84	7535·90	2·601	0·107
	St × trap	48	11172·81	7537·42	4·123	0·050
	t + Sx	53	11158·21	7537·90	4·602	0·039
	t	52	11163·76	7539·56	6·256	0·017
	St + t + Sx	54	11158·19	7539·93	6·633	0·014
	Trap	42	11183·04	7540·17	6·869	0·013
	St + t	49	11163·65	7541·53	8·232	0·006
(b) Survival rates (Φ)	(U × prev) + I + a2 + Sx	34	11195·53	7524·10	0	0·232
	a2 + Sx	32	11204·02	7525·71	1·611	0·120
	t + a2 + Sx	39	11182·89	7525·83	1·725	0·113
	prev + t + a2 + Sx	40	11180·13	7526·02	1·915	0·103
	a2	31	11208·75	7526·84	2·736	0·068
	t + a2	38	11188·33	7527·42	3·315	0·051
	a2 × Sx	33	11204·00	7527·72	3·621	0·044
	St + a2 + Sx + t	40	11182·81	7527·80	3·701	0·042
	t + a2 × Sx	40	11182·87	7527·84	3·739	0·041
	(St + prev) + a2 + Sx	41	11180·10	7528·04	3·939	0·037

The top ten models in each candidate set are shown. k = number of parameters; *w* = model weight. † Most parsimonious recapture rate model retained for modelling survival rates. See Table 2 for model notation.

(Table 3a); with estimates showing that uninfected birds had higher recapture rates than infected birds (Fig. 2a). However, there was also very strong support for a disease effect in recapture rates in the species-specific analysis, indicating that recapture rates varied not only between uninfected and infected birds, but also between birds infected with different malaria species (Table 4a). Although the top model in the species-specific analysis allowed recapture rates to vary between all three states, two pieces of evidence indicate that the strong support for a disease effect was entirely because of differences between the different malaria species. First, models in which the recapture rates of the uninfected state

Table 4. Summary results of the multievent mark–recapture analysis modelling the effect of *Plasmodium relictum* ('R' Clade) and *P. circumflexum* ('C' Clade) infections on recapture and survival rates of blue tits

Parameter	Model	*k*	Deviance	QAICc	ΔQAICc	w_i
(a) Recapture rates (p)	St + boxD*	74	10499·93	7134·63	0	0·374
	St + boxD with St = UR v C	73	10503·78	7135·11	0·481	0·294
	St + boxD with St = UC v R	73	10505·54	7136·28	1·657	0·163
	St + boxD + Sx	75	10499·36	7136·72	2·098	0·131
	boxD	72	10514·86	7140·42	5·789	0·021
	St + boxD with St = U v RC	73	10514·10	7141·99	7·361	0·009
	St × boxD	78	10507·12	7147·75	13·123	0·001
	St	72	10531·53	7151·53	16·904	0·000
	St + trap	73	10530·31	7152·79	18·168	0·000
	St + Sx	73	10531·39	7153·52	18·893	0·000
	St + edge	74	10530·35	7154·90	20·277	0·000
(b) Survival rates (Φ)	(St + t) + (R/C × a2) + (U × prev)	50	10553·35	7137·10	0	0·434
	(St + t) + a2 + (U × prev)	58	10554·90	7138·13	1·032	0·259
	(St + t) + a2	57	10562·61	7141·21	4·108	0·056
	(St + t) + a2 + prev	60	10553·68	7141·44	4·343	0·049
	(St + t) + (R/C × a2)	58	10559·91	7141·48	4·373	0·049
	(St + t) + a2 *with St = UR v C*	56	10567·63	7142·50	5·399	0·029
	(St + t) + (St × a2)	59	10558·52	7142·61	5·504	0·028
	(St + t) + a2 + Sx	58	10562·54	7143·23	5·865	0·023
	(*St + t*) + *a2 with St = UC vs. R*	56	10569·10	7143·48	6·126	0·020
	(St + t) + (St × a2) + Sx	56	10558·41	7144·599	7·497	0·010

The top ten models in each candidate set are shown. *k*, number of parameters; *w*, model weight.
*The most parsimonious recapture rate model retained for modelling survival rates. See Table 2 for model notation.

were combined with those of either R-Clade or C-Clade states received equivalent support to the top model, whereas a model in which R-Clade and C-Clade states were combined received substantially less support (Table 4a). Second, the 95% confidence interval for the effect of C-Clade state relative to R-Clade state on recapture rates did not include zero [average effect size on the logit scale (±95% CI) = 1·03 (0·49, 1·42)], whereas those for either of the infected states relative to the uninfected state did [R-Clade relative to the uninfected state = −0·29 (−0·27, 0·21), and C-Clade relative to the uninfected state = 0·41 (−0·01, 0·81)].

Recapture rates for individuals infected with R-Clade malaria were lower than those of individuals infected with C-Clade malaria, while the recapture rates of uninfected individuals were intermediate to the two infected states (Fig. 2b). The variation in recapture rates between malaria species could not be attributed to the proximity of individuals to the woodland edge, nor to spatial variation in nestbox density (Table 4a). However, there was strong support for an additive effect of nestbox density on recapture rates (Table 4a). In this population, blue tits were more likely to be captured in areas of greater nestbox density (Fig. 2b).

There was some support for an additive effect of sex in recapture rates in the combined-species analysis (Table 3a) with estimates showing that females were more likely to be captured than males (Fig. 2a). However, in the species-specific analysis sex differences in recapture rates were no longer strongly supported (Table 4a). In both analyses, models with yearly variation in recapture rates were not well supported, indicating that encounter rates were relatively constant throughout the study. There was also little support for trap dependence in recapture rates in either analysis (Tables 3a and 4a).

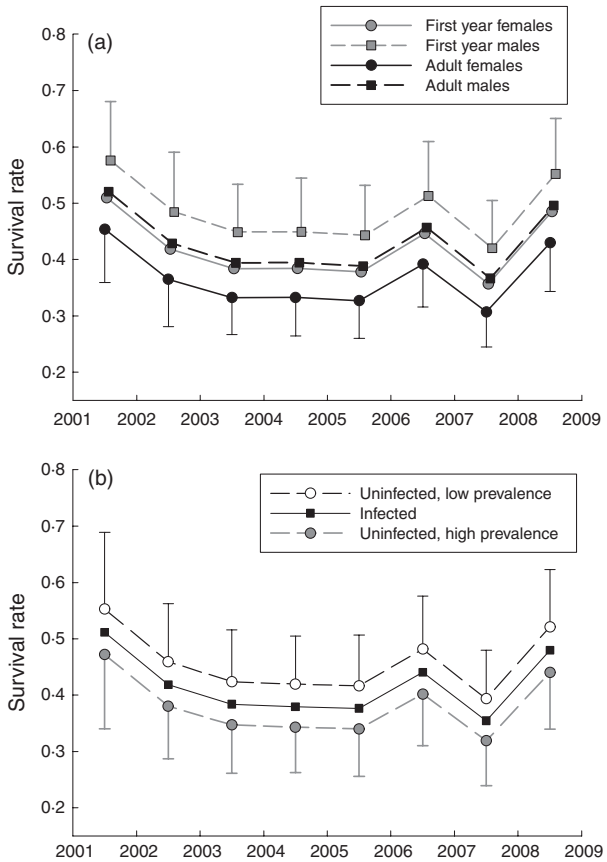

Fig. 3. Results of combined *Plasmodium* multievent mark–recapture modelling of survival rates showing (a) survival rates of blue tits as a function of host age and sex and (b) the effect of disease risk (population prevalence) on survival of blue tits. Estimates are model averaged means ± 95% CI. To clarify patterns, not all error bars are shown.

APPARENT SURVIVAL RATES

In the combined-species analysis, the most parsimonious model in the candidate set included only the effects of age and sex on host survival, with equivalent support for models with either constant or yearly variation in survival rates (Table 3b). Adult survival rates were on average lower than those of yearlings, while female survival rates were lower than those of males (Fig. 3a). In this analysis, there was little support for models in which survival rates varied between disease states (Table 3b), suggesting that malaria infections do not impact greatly on host survival. However, as discussed earlier, we are likely to have missed most acute infections in this population. If acute infections carry fitness costs, then survival rates should be lower in areas where there is a higher force of infection (and thus greater risk of infection). Results showed strong support for models in which survival rates of individuals varied between areas of high and low malaria prevalence, with more weight for the model in which only the survival rate of uninfected individuals varies with disease prevalence (Table 3b). This result thus suggests that acute malaria infections do cause significant mortality for hosts in this population. Estimates from this model show that survival rates of uninfected individuals were on average 17% lower in high than in low-prevalence areas (Fig. 3b). This result did not appear to be driven by an underlying correlation between high habitat quality and low *Plasmodium* prevalence as low-prevalence sites contained far fewer Oak trees than did high-prevalence areas ($t = -3{\cdot}155$, df = 799, $P < 0{\cdot}001$). A consequence of missed infections (those occurring outside of our sampling periods) is that parameter estimates for the uninfected state will be biased, because some individuals classified as uninfected will have become infected between sampling occasions. This bias likely explains why recapture and survival rates for uninfected individuals were found to be intermediate to those of individuals infected with the two *Plasmodium* species.

In the species-specific analysis, as a result of the paucity of information on infection status in the early years of this study (no malaria testing in 2002 and species diagnoses for only five individuals captured in 2003), survival rates could not be estimated in 2003 [confidence intervals for these estimates were either abnormally large (from 0 to 1) or small (equal to zero)]. In contrast to the combined-species analysis, there was strong support for time variation in survival rates in this analysis

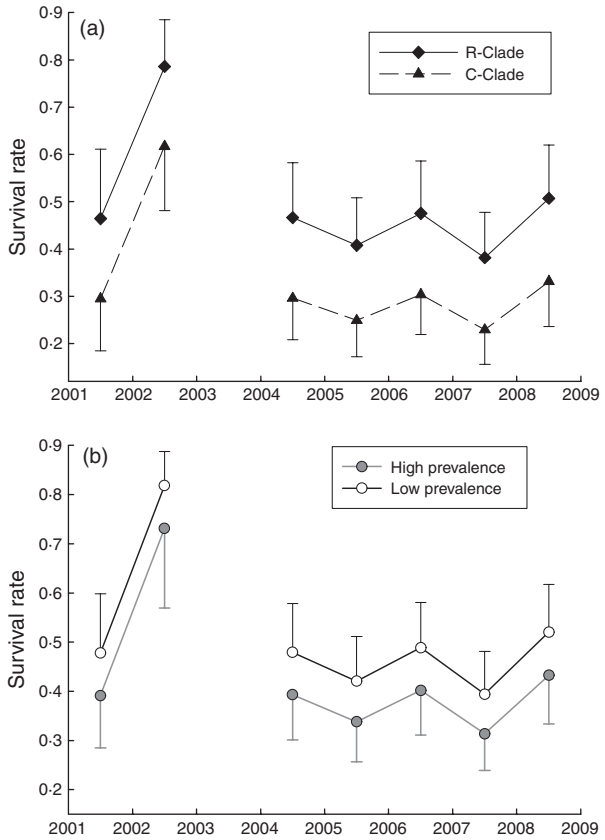

Fig. 4. Results of species-specific multievent mark–recapture analysis of survival rates showing (a) effect of *Plasmodium relictum* (R-Clade) or *P. circumflexum* (C-Clade) infection on the survival rates of blue tits; and (b) effect of disease risk (population prevalence) on survival rates of uninfected blue tits. Estimates are model averaged means ± 95% CI.

that appeared to be driven by a single particularly high survival rate of individuals from 2002 to 2003 (Table 4b, Fig. 4). Also in contrast to findings with *Plasmodium* species combined (Table 3b), results of the species-specific analysis revealed very strong support for a disease effect on survival rates (all the top models in the candidate set included an effect of disease state on survival rate; denoted 'St' in Table 4b), indicating that the two malaria species differed in their impacts on host survival. Models in which the survival rates of all three states differed received more support (ΔQAICc > 5) than models in which any of the states were combined (denoted 'UR vs. C' or 'UC vs. R' in Table 4b). However, as reported for recapture rates above, the only significant effect of disease state on survival rates was that of C-Clade relative to R-Clade state (average effect size on the logit scale (±95% CI) = −0·708 (−1·46, −0·35). Survival rates of individuals infected with C-Clade malaria (*P. circumflexum*) were on average 31% lower than the survival rates of individuals infected with R-Clade malaria (*P. relictum*) (Fig. 4a). Again, as detailed earlier, models in which the survival rate of uninfected individuals varied between high and low-prevalence areas were also strongly supported here (Table 4b), with the reduction in the survival rates of uninfected individuals in high-prevalence areas similar to that in the species-combined analysis (Fig. 4b).

In contrast to the combined-species analysis, the best-supported models in the species-specific analysis included only an effect of host age and not sex on survival rates (Table 4b). Overall adult survival rates were 14% lower than the survival rates of yearling birds, which was similar to the difference observed in the combined-species analysis. There was also some support for models in which this age effect was restricted to infected states only (equivalent support for models '(St + t) + a2' and '(St + t) + (R/C × a2)'; Table 4b), suggesting differential impacts of malaria infection on adults and yearlings. Model selection revealed little support for models in which survival rates varied with the distance individuals were from the woodland edge. There was also no difference in the average within-site movement of uninfected individuals and individuals infected with different malaria species ($F_{(2,793)} = 1{\cdot}170$, $P = 0{\cdot}3118$).

Discussion

Understanding the ecological and evolutionary implications of parasites requires knowledge of their effects on hosts in natural populations. Avian blood parasites have long served as a model system for investigating host–parasite interactions yet their impacts on host fitness in endemic conditions remain very poorly understood. Difficulties in detecting the effects of endemic malaria infections can be attributed to infections being comprised of multiple cryptic species, to the fact that the majority of individuals in natural populations harbour only chronic infections and to sampling inequalities between disease states. In this study, by incorporating information on parasite diversity and spatial variation in disease risk, and by accounting for state-dependent detectability in a mark–recapture framework, we were able to demonstrate effects suggesting that both acute and chronic malaria infections entail substantial fitness costs for hosts in a population where transmission is endemic. More importantly, our results revealed that the two malaria species within our population impacted in contrasting ways on host fitness components and will thus impose very different selective pressures on hosts in this population.

The costs of acute *Plasmodium* infections can be severe for naive hosts in regions where avian malaria has been recently introduced (Atkinson & Samuel 2010) but have never been quantified in natural populations where hosts have co-evolved with these parasites. In this study, blue tits were sampled for infection status annually, at a fixed point in their annual cycle, such that many within-year infections were presumably missed, particularly as peak *Plasmodium* transmission may be outside our sampling period (Cosgrove *et al.* 2008). Thus, like many studies investigating host–parasite interactions in avian malaria systems (Bensch *et al.* 2007; Marzal *et al.* 2008), we could not directly measure the acute effects of malaria infection on hosts. Nonetheless, by explicitly incorporating information on the spatial variability of the force of infection within our population, we were able to

demonstrate that survival rates of individuals, particularly uninfected individuals, in high-prevalence areas were considerably lower than those of individuals in low-prevalence areas. This result did not appear to be driven by correlated differences in habitat quality (at least in terms of the abundance of oak trees). Moreover, that uninfected individuals suffered a greater survival cost than infected individuals in high-infection-risk areas argues against this pattern being a result of other spatially correlated confounding factors or differential emigration rates (see also the discussion below). We suggest this result provides evidence of an acute effect of *Plasmodium* infection. However, we cannot discount the possibility that this result might also reflect differential settlement patterns of high- and low-quality individuals (individuals with intrinsically higher survival rates prefer low-prevalence areas). Confirmation that acute *Plasmodium* infections entail appreciable mortality costs for hosts in this wild population will ultimately require a much finer temporal sampling scale (days to weeks): a logistically difficult task to implement.

Acknowledging the potential for *Plasmodium* species to differ in their effects on hosts greatly improved our ability to detect impacts of chronic infections on host fitness in this species. With the addition of species-specific information, model selection revealed that survival rates of individuals infected with *P. circumflexum* were substantially lower than individuals infected with *P. relictum*; explaining the absence of a disease state effect on survival rates in the combined-species analysis. That the combined effects of multiple malaria species may mask the underlying impacts of any one species could be responsible for the failure of many studies to detect fitness effects of chronic malaria infections in the wild (e.g Bensch *et al*. 2007; Ortego *et al*. 2008; Marzal *et al*. 2008). Indeed, this is the first study in a wildlife species to demonstrate differences in survival rates between individuals of a single host species infected with different malaria species (though such effects are apparent in humans, Clark & Schofield 2000).

There is little reason to suspect that these observed differences in apparent survival rates were an artefact of differential emigration rates: survival rates did not vary with the location of individuals within study site, as might be expected if edge-dwelling individuals were more likely to emigrate. Further, within-site dispersal distance (an index for emigration propensity) did not differ between uninfected individuals and individuals infected with either *P. relictum* or *P. circumflexum*. Instead, the considerable difference in survival rates between individuals infected with *P. relictum* and *P. circumflexum* suggests that *P. circumflexum* is more virulent than *P. relictum*. Although it is not entirely unexpected that the virulence of different malaria species may vary within a single host species, few studies to date have examined such effects (Atkinson & van Riper 1991), particularly in wild populations. Although the generality of the pattern observed here remains to be seen, an evolved lower virulence in *P. relictum* might explain the much broader documented host range and larger global distribution of this species compared with *P. circumflexum* (Bensch, Hellgren & Perez-Tris 2009; Hellgren, Perez-Tris & Bensch 2009).

Alternatively, infections with different *Plasmodium* species may induce varied behavioural or physiological changes in hosts that could either increase the exposure of hosts to other sources of mortality (e.g. predation, Moller & Nielsen 2007) or predispose hosts to secondary infections (Poulin 1994). Indeed, if individuals infected with *P. circumflexum* suffer multiple parasite infections, this may explain the observed higher virulence of *P. circumflexum* malaria relative to *P. relictum*, as within-host competition between parasites is assumed to select for higher virulence (Choisy & de Roode 2010). The possibility that hosts infected with *P. circumflexum* experience lower survival rates as a result of either greater predation pressures or greater parasite burdens has important implications for our understanding of the way these parasites can shape host populations and warrants further investigation.

Another plausible explanation for differences in survival rates between individuals infected with different malaria species is if infection triggers a change in reproductive output that results in increased survival, owing to reduced costs of reproduction (Sheldon & Verhulst 1996). In this study, recapture rates of individuals infected with *P. relictum* were significantly lower than for individuals infected with *P. circumflexum*. As only birds that were actively breeding were captured in this study, one explanation of this pattern is that infection with *P. relictum* causes early breeding failure in hosts. Support for this suggestion is provided by two recent studies. The first, in a neighbouring population of blue tits to that studied here, found that infections with *Plasmodium* parasites (predominantly *P. relictum* in this population) were associated with nest abandonment prior to hatching (Knowles, Palinauskas & Sheldon 2010a). Second, as shown by Knowles, Wood & Sheldon (2010b), infection by *P. relictum* exacerbates the cost of increased brood size in terms of reproductive output in blue tits. Hence, as has been shown for other avian blood parasites in other hosts (Marzal *et al*. 2005; Ortego *et al*. 2008), it appears that chronic *P. relictum* infections entail a number of significant reproductive costs for breeding blue tits. Individuals infected with *P. relictum* were observed to have higher survival rates than individuals infected with *P. circumflexum*, suggesting that the diversion of energy away from reproduction might benefit their long-term survival. A similar pattern has been observed for rodents infected with cowpox virus, whereby higher survival rates of infected hosts relative to uninfected hosts were attributed to the fact that infected rodents expend less reproductive effort because infection caused individuals to delay reproduction until the following breeding season (Telfer *et al*. 2002, 2005).

Our results showing that recapture and survival rates differed in opposing ways in individuals infected with the two malaria species raise the possibility that blue tits modify their reproductive strategies in a manner consistent with the theoretical predictions regarding adaptive host life-history responses to parasitism (Perrin, Christe & Richner 1996;

Agnew, Koella & Michalakis 2000). Individuals infected with *P. relictum,* which may have evolved lower pathogenicity, are able to invest to a greater degree in immune resistance (as infection compromises their current reproduction effort) and thus experience higher survival rates. Conversely, as *P. circumflexum* appears more virulent, individuals may respond to infection by maximize their current reproductive effort when survival prospects are challenged. Experimental work is clearly needed to conclusively evaluate the role of these malaria species in the resource allocation strategies employed by blue tits.

A key finding from this study was that recapture rates differed substantially between uninfected and infected individuals and between individuals infected with different *Plasmodium* species. We also found clear evidence that capture probabilities of birds varied independently as a function of nestbox density. These effects were very well supported and large: the probability of capturing a bird infected with *P. circumflexum* in an area with high density of nestboxes was 68% greater than for a bird infected with *P. relictum* in an area of low nestbox density (Fig. 2b). Such variation will be a very important source of bias in prevalence estimates and estimates of demographic parameters in studies that have not used mark–recapture methods to account for differences in detectability. For example, correcting species-specific prevalence estimates for state-dependent recapture rates in our population results in prevalence estimates that are on average 33% higher for *P. relictum* and 22% lower for *P. circumflexum* (see Table 1). Such capture heterogeneity among individuals infected with different malaria species will have profound implications for studies in which 'apparent' prevalence is used to compare the effects of different malaria species, (Crespin *et al*. 2008).

This study is one of the few to have demonstrated that malaria infections have significant negative consequences for host fitness in a wild population where transmission is endemic. More importantly, this study revealed that different malaria species can have very different effects on host fitness components in a single host species. Crucially, these results were only apparent with the inclusion of information on the diversity of *Plasmodium* infections within our population, as well as information on the spatial variation in risk of infection within the study site. This highlights the importance of considering genetic variability among parasites and the ecological context of the host–parasite interaction when studying the consequences of endemic infections on natural populations. The magnitude of the impacts of chronic *P. circumflexum* infections on survival rates, the strong indications of acute effects of *Plasmodium* infection, together with the inferred impact of *P. relictum* infections on host reproduction clearly indicate that malaria infections should no longer be dismissed as having negligible effects on their host in endemic regions (Ricklefs & Outlaw 2010). Indeed, that these two malaria species differ so greatly in their effects on host fitness indicates they will likely impose very different selective pressures on hosts in this population. Investigating how such differential selection pressures contribute to the evolution of genetic aspects of host resistance and host life-history strategies is a fruitful area for further research.

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Supporting Information

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

Table S1. Diversity of avian malaria lineages in the Wytham Woods blue tit population.

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